Mycelial and hyphal interactions in holocoenocytic basidiomycotina

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Award date:
1986

Awarding institution:
University of Bath

MYCELIAL AND HYPHAL INTERACTIONS IN HOLOCOENOCYTIC BASIDIOMYCOTINA

submitted by Antony Martyn Ainsworth

for the degree of Ph.D.

of the University of Bath

1986

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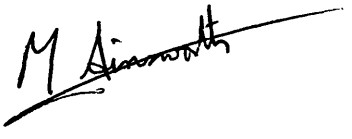

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Frontispiece. Wild basidiomata in *Stereum*, *Phanerochaete* and *Coniophora*. (a) *S.* "*rameale*". (b) *S. sanguinolentum*. (c) *S. subtomentosum*. (d) *S. insignitum* (life size). (e) *S. rugosum*. (f) *S. gausapatum*. (g) *S. hirsutum*. Overleaf, (h) *P. velutina*. (i) *P. laevis* (twice life size). (j) *C. puteana*. Coin diameters are 2.2cm in (g) and 2cm in the remainder.

Photographs (i) and (j) were taken by C. G. Dowson and R. C. Dryden respectively.


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ACKNOWLEDGEMENTS

I cordially thank the Science and Engineering Research Council for financial support and my patient supervisor Dr. Alan Rayner for intellectual support, enthusiastic guidance and exciting discussion.

I am indebted to the School of Biological Sciences, University of Bath, for providing facilities and technical assistance and to Drs. Robert Aylmore and the late Norman Todd for instruction in the use of the microculture chamber featured in Chapter 8 of this thesis.

I am also grateful to Dr. Derek Reid for identifying my first collection of S. subtomentosum, to Mr. Peter Jewell and the Network ladies for word processing and to Drs. Lynne Boddy and David Coates for showing me the ropes.

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SUMMARY

Multinucleate hyphal compartments with multiple clamp connections in basidiospore-derived primary mycelia have led to Stereum, Phanerochaete and Coniophora being generally regarded as homothallic genera. However, this project revealed two breeding strategies, outcrossing and non-outcrossing, which can co-exist within a single taxonomic species. They were analysed in terms of heterogenic and homogenic incompatibility system interplay whose respective rejecting and accepting tendencies regulate genetic exchange via non-self fusion between genetically dissimilar hyphae.

Direct phase contrast microscopic hyphal fusion studies in P. velutina revealed three response types, nuclear division/septation, septal erosion/nuclear migration and hyphal rejection. Macroscopic interactions in plate culture were envisaged as aggregations of these different types occurring in varying proportions during a mycelial interaction, including sequential occurrence in a single fusion compartment.

Primary mycelial outcrossing was interpreted as the homogenic incompatibility system temporarily overriding the heterogenic incompatibility system. This non-self acceptance was microscopically evident in septal erosion/nuclear migration and macroscopically yielded secondary mycelium on morphological and interactive criteria. Secondary mycelial establishment was detected within S. hirsutum, S. gausapatum, S. rugosum, S. insignitum, P. velutina, P. laevis and C. puteana according to

a unifactorial diaphoromictic system and was resolved into three possible components, viz. access migration, acceptor migration and stabilization. Morphological attributes of access and its operation in mating-type incompatible combinations suggested an evolutionary link with the B-factor of bifactorial diaphoromixis.

Non-outcrossing was interpreted as heterogenic incompatibility maintaining the genetic integrity of sporulating primary mycelia in the absence of an overriding homogenic system, possibly resulting from the activation of normally unexpressed mating-type factors. This facilitated natural population structure analysis in S. hirsutum, S. sanguinolentum, S. "rameale" and S. subtomentosum. Their primary mycelial interactions resembled those of secondary mycelia in that both involved rejection of donor nuclei, but they differed in that nuclear donation to conspecific outcrossing primary mycelia was not achieved. It is suggested that non-outcrossing genotypes are sequestered components of an ancestral meiotically recombining gene pool.

PART I

MYCELIAL INTERACTIONS

CHAPTER 1 INTRODUCTION

BACKGROUND - THE TERMINOLOGY AND SIGNIFICANCE OF MYCELIAL INTERACTIONS IN THE BASIDIOMYCOTINA

HOMOTHALLISM AND HETEROTHALLISM

Blakeslee showed that two mycelia of Rhizopus stolonifer (R. nigricans) (Zygomycotina), each originating from a single spore, must interact in order to complete the sexual cycle (e.g. Blakeslee, 1906). Moreover, he discovered that this and several other mucoraceous species comprised two physiologically (but not morphologically) distinctive kinds of mycelia and significantly that it was only those interactions involving both kinds which resulted in fertility. Blakeslee termed such cross-fertility heterothallism and distinguished it from homothallism. The latter was applied to species with a single kind of mycelium which was capable of sexual reproduction (zygospore development) when cultured in isolation from a single spore, i.e. self-fertile.

Following the establishment of the homothallism/heterothallism terminology, numerous attempts were made to accommodate other fungi, including the Basidiomycotina, within these two categories. Sexuality in those basidiomycetes which lack sex organs will now be considered, but first it is necessary to define the life cycle phases described by the terms primary and secondary mycelium in the sense in which they are used throughout this thesis, unless specifically qualified. The

primary mycelial phase begins at spore germination, whereas the secondary mycelial phase begins when a morphologically stable and distinct mycelium develops from a pair of interacting mycelia.

Fruiting *per se* was one of the earliest criteria for a successful heterothallic interaction (see Bose, 1934), but later the fruiting of primary mycelia was taken as evidence for homothallism, as with *Coprinus sterquilinus* (Lendner, 1920; cited by Whitehouse, 1949). Kniep (1920; cited by Whitehouse, 1949) discovered that primary mycelia of *Schizophyllum commune* sometimes fruited when cultured alone and progeny analysis revealed the presence of the one parental mycelial kind *sensu* Blakeslee. On other occasions fruiting occurred on secondary mycelia, but the resulting progeny were of more than two kinds. This marked a significant departure from Blakeslee's concept of a species comprising -/+ kinds of mycelia, which he had used interchangeably with male/female although somewhat arbitrarily. Furthermore, Kniep undermined the assumption that sex was actually occurring in those forms designated as homothallic by a cytological study of sporogenesis in fruiting primary mycelia of *S. commune*. This, he concluded, was an entirely asexual (mitotic) process, albeit in a fungus that was demonstrably cross-fertile. Nevertheless, the terms heterothallic and homothallic *sensu lato* were retained within the Basidiomycotina, respectively distinguishing those which are capable of forming secondary mycelia (outcrossing) from those whose primary mycelia are incapable of initiating the secondary phase (non-outcrossing).

Onset of the secondary phase is accompanied by various physiological and morphological changes, such as increased radial

extension rate and aerial mycelial density, reduced hyphal branching angle and the production of clamp connections, i.e. short hyphal arches connecting neighbouring compartments of a subtending hypha. Cytologically, the latter had been associated with the presence of binucleate compartments in forms whose primary mycelial compartments were uninucleate (e.g. Kniep, 1920; cited by Raper, 1966a). In its turn, the relatively quick and simple detection of clamp connections and/or binucleate compartments became the criterion for distinguishing between homothallism, if occurring in primary mycelia, and heterothallism if occurring only in secondary mycelia (e.g. Koltin, Stamberg & Lemke, 1972).

Care should be taken when interpreting the confusing literature concerning secondary mycelia and their relationship to homothallism and heterothallism. For example, Buller (1941) equated the secondary phase with a clamp-bearing mycelium and Kühner (1977) interpreted the binucleate basidiospores of Typhula micans as the beginning of the secondary phase of a homothallic life cycle which lacked a preceding primary mycelial stage. The importance attached to, and routine use of, clamp connections in this respect, together with the concomitant neglect of more lengthy determinations, was not however without criticism. Although largely unheeded, Oort (1929; cited by Bose, 1934) warned that if used alone without supportive evidence from progeny analysis and changed mycelial morphology, "the occurrence of clamp connections can only be of restricted use".

Several other theories of sex, criteria and types of heterothallism and homothallism were proposed (reviewed by Buller, 1941; Whitehouse, 1949; Burnett, 1956; Raper, 1965; Esser

& Kuenen 1967) before the concept of incompatibility gained widespread acceptance (Esser, 1966; Esser & Kuenen, 1967). Thus a genotypically determined failure of nuclear fusion (karyogamy), and hence of meiospore production, was ascribed to the interaction of incompatibility factors which ultimately controlled crossing relationships within a species.

INCOMPATIBILITY SYSTEMS

Homogenic incompatibility

This term, although logically inferior to heterogenic compatibility (Rayner et al., 1984) was introduced to describe fungal breeding systems and defined by Esser (1966) as a "restriction of mating competence due to like alleles of one or more loci". If one factor, or locus sensu Esser (1966), was involved per primary mycelium, sibs (primary mycelia derived from the same basidioma) could be assigned one of two possible mating-types (bipolar sexuality), whereas if two unlinked factors per primary mycelium were involved, sibs fell into four mating-type classes (tetrapolar sexuality). There is also a report of an unlinked three factor octopolar system in Psathyrella coprobia (Jurand & Kemp, 1973), although doubts have been expressed about the strength of supporting evidence (Day, 1978). It should be stressed that in all three systems of polarity, two mycelia show degrees of homogenic incompatibility if they are homozygous for any mating-type factor.

An improved and comprehensive scheme of breeding system classification was introduced by Burnett (1956) which, although complex, made several important distinctions. For example,

outcrossing (heteromixis) was subdivided into dimixis and diaphoromixis, respectively with two and multiple mating-type specificities in the breeding population. Diaphoromixis was then further qualified by the descriptors unifactorial (bipolar), bifactorial (tetrapolar) and trifactorial (octopolar) (Burnett, 1975). Terms which imply a number of mating-type factors within a primary mycelial genome seem preferable to those specifying alleles or loci in view of Papazian's (1951) generation of new factor specificities by intra-factor recombination in S. commune, Takemaru's (1961) detection of non-parental factors in other bifactorial forms and similar findings in unifactorial Thanatephorus cucumeris (Rhizoctonia solani) (Anderson et al., 1972) and Agaricus bitorquis (Raper, 1976).

Turning now to the terminology of the factors themselves, those of unifactorial Basidiomycotina could be unambiguously labelled A factors and their specificities written in serial equivalence as A1, A2, A3...., etc. (Raper, 1966a). Initially, the two factor series of bifactorial forms were arbitrarily designated as A and B factors and hence no correspondence should be inferred between different species and authors. The situation was temporarily ameliorated following Papazian's (1950) studies on S. commune and Fulton's (1950) work on Cyathus stercoreus. Both recognized fundamental differences in the morphogenetic sequences operating in hemicompatible matings, i.e. involving different factors of one of the bifactorial series only. In the hemicompatible pairings A1B1 x A1B2 and A1B1 x A2B1, the former was called hemicompatible-A, having unlike B-factors (B≠) and hence an operative B-on morphogenetic sequence, whereas the latter was called hemicompatible-B, having unlike A-factors (A≠)

and an operative A-on sequence. Subsequently, the more consistent and distinctive type of hemicompatible interaction was attributed to the action of the B-on sequence which could be generally inferred from the presence of gnarled and knobby hyphae, irregularly beset with short lateral branches (Raper, 1966a). This was macroscopically recognizable by a sparse and "flat" morphology, i.e. with little aerial mycelium (Papazian, 1950), or in the terminology of Fulton (1950) "blotchy", and/or with mutually restricted marginal hyphal extension, e.g. Vandendries & Brodie's (1934) observations of Psathyrella candolleana (Hypholoma candolleanum) and Coriolus zonatus.

Generally, the A-on sequence was morphologically less consistent in its macroscopic expression, but could sometimes be associated with a region of sparse and flat mycelium at the mycelial confrontation zone. This was described as "barrage sexuel" by Vandendries, e.g. in Pleurotus ostreatus (Vandendries, 1933; Terakawa, 1957), and particularly in Lenzites betulina (Vandendries, 1934). However, the application of the term barrage to similar phenomena occurring in various other types of bifactorial pairings, e.g. hemicompatible-A interactions in Armillaria mellea (Korhonen, 1978a) and fully mating-type compatible interactions in Polyporus spp. (Hoffmann & Esser, 1978), coupled with an absence of barrage in an increasing number of bifactorial progeny sets, led to a diminution of its diagnostic importance (Raper, Krongelb & Baxter, 1958; Esser & Meinhardt, 1984). Confusingly, it has also been loosely applied within the Basidiomycotina to ridges of bulked aerial mycelium at the confrontation zone (Adams, 1982) and many other types of visible zones of aversion or demarcation between mycelia, e.g.

between field isolates (Brodie, 1935), unifactorial sibs (Burnett, 1956) and to interactions in the Ascomycotina (Esser, 1965) and Deuteromycotina (Burnett, 1976). However, microscopic studies within those members of the Basidiomycotina where clamp connections accompanied secondary mycelial establishment revealed that the A≠ function was generally associated with the production of pseudoclamps in the interaction zone (Raper, 1966a). In the absence of phase contrast microscopic studies, these structures could be difficult to distinguish from true clamp connections, although they fundamentally differ in that the arching hyphal branch (hook cell) fails to fuse with the subtending hypha. As a result, some studies in which the search for clamp connections was confined to the interaction zone led to some species being classified as both unifactorial and bifactorial by different workers. This was resolved by Aschan (1954) and Takemaru (1961) who showed that the bifactorial nature of certain species could be unmasked, provided that clamp connections were additionally sought in locations distal to the interaction zone.

From 1950 it was generally accepted that the A-factor controlled nuclear pairing and the formation and septation of hook cells, whereas the B-factor controlled nuclear migration and hook cell fusion (Fulton, 1950; Raper, 1966a). However, recent anomalous results have shown that this assumption is no longer tenable. Bruehl, Machtmes & Kiyomoto (1975) were unable to detect regular pseudoclamp formation or nuclear migration in mating-type incompatible sib pairings of Typhula spp. and so could not distinguish between the A-on and B-on morphogenetic sequences. A similar equivalence of mating-type factors was proposed by Meinhardt, Epp & Esser (1980) for Agrocybe

cylindracea (A. aegerita), but this was withdrawn after the study was extended (Meinhardt & Leslie, 1982). Distinct but different attributes of the A- and B-factors were suggested by Butler (1972) in Coprinus disseminatus and Kemp (1980a) in other members of this family. In C. disseminatus it was suggested that one factor, A, controlled the whole clamp formation process, whilst the other, B, controlled nuclear migration. However, this was based on a study of clamp connection formation which occurred at rates comparable with nuclear migration in other species (putative A≠ B≠ pairings), or at lower rates similar to or less than the secondary mycelial radial extension rate (putative A≠ pairings). No data were presented for B≠ pairings and so the requirement for B≠ or A≠ B≠ in the faster clamp-forming process was not clarified. Kemp (1980a) described a similar scheme in the Coprinus patouillardii group in which secondary mycelia either emerged from the junction of mated primary mycelia (one factor ≠), or became established after extensive bilateral nuclear migration (both factors ≠). As expected, progeny of the former secondary mycelial type showed a unifactorial sib mating pattern, whereas those of the latter type showed a bifactorial sib mating pattern.

It has been repeatedly stated that diaphoromixis, by virtue of its multiple specificities, shifts the balance which exists between dimictic outbreeding (non-sib mating-type compatibility) and inbreeding (sib mating-type compatibility), such that the former is promoted whilst the latter is unchanged (unifactorial), or reduced (bifactorial and trifactorial), (e.g. Mather, 1942; Raper, 1966b; Burnett, 1975; Webster, 1980). However, care must be taken when interpreting the literature

which refers to specific percentages of inbreeding/outbreeding, spatial distribution, estimation of the number of mating-type factors in the potentially breeding population based on that present in small samples and calculations of outbreeding efficiency (e.g. Eggertson, 1953; Raper, 1966a; Stamberg & Koltin, 1973; Ullrich, 1977; Koltin, 1978). The sample sizes, methods and assumptions (Whitehouse, 1949) contributing to such estimates, e.g. that incompatibility factors were equally frequent and randomly distributed, have recently been questioned in view of the previous lack of information on the ecology and population structure of the Basidiomycotina (e.g. Rayner & Todd, 1982a; Cooke & Rayner, 1984; Williams & Todd, 1985). Moreover, in a study of homogenic incompatibility within Laccaria, Fries & Mueller, (1984) showed that there were significant departures from the expected 1:1:1:1 ratio of mating-types for cultured sets of bifactorial sibs. Similar departures were reported in the unifactorial Phlebia radiata (Boddy & Rayner, 1983) which would tend to decrease inbreeding if the situation obtained in nature. However, a study of unifactorial Agaricus macrosporus (Elliott, 1978) emphasized the requirement for numerous large progeny sets to prevent unwarranted speculation. He analysed two progeny sets of 20 and 39 sibs and discovered the ratios of mating-types were 16:4 and 20:19 respectively.

Heterogenic incompatibility

Studies on Podospora anserina (Ascomycotina) enabled Esser and coworkers to detect a contrasting incompatibility system (I.S.) incorporating allelic, non-allelic and cytoplasmic mechanisms which manifested itself between isolates of different geographical origin. This led to the general definition, "heterogenic incompatibility is brought about by genetic elements

which cannot exist in close proximity to each other, i.e. neither in one nucleus nor in a common cytoplasm" by virtue of "at least a genetic difference of one single gene or one cytoplasmic factor". In an extensive review of heterogenic I.S. in fungi, plants and animals, Esser & Blaich (1973) drew attention to the difficulty that may arise when distinguishing sterility based on gross genetic differences and heterogenic incompatibility based on specific small genetic differences. They cited the nonreciprocal incompatibility of P. anserina interracial crosses as providing the most convincing evidence for a true heterogenic I.S., to which must be added the cytoplasmic nonreciprocal incompatibility of small brown planthoppers Laodelphax striatellus from different Japanese sites (Noda, 1984) and American strains of the mosquito Culex pipiens (Barr, 1980). It followed that incompatibility studies on samples of natural populations would be expected to increase the probability of sufficient genetic differences between isolates to reveal the heterogenic I.S.. Indeed, the routine use of relatively inbred laboratory strains with a large degree of isogeneity probably led to the comparatively late discovery of this recognition phenomenon (Esser, 1971). Moreover, under such conditions it could also be masked by forced heterokaryosis (presence of nuclei of unlike genotype in a single mycelium that is not necessarily stable) between nutritionally complementary (auxotrophic mutant) homokaryons (mycelia containing nuclei of a single genotype) (Caten & Jinks, 1966).

Several other terms have also been accommodated within the heterogenic I.S., including i) protoplasmic incompatibility in P. anserina (Labarère, Bégueret & Bernet, 1974) and in

Neurospora crassa (Ascomycotina) (Garnjobst & Wilson, 1956) which was used to indicate that the incompatible response followed hyphal fusion (anastomosis); ii) heterokaryon incompatibility in Emericella (Aspergillus) nidulans (Ascomycotina), a taxon which revealed numerous component heterokaryon-compatibility (h-c) groups (Grindle, 1963) which, although resembling the incompatible geographical (allopatric) races of P. anserina, significantly occurred in overlapping geographical areas (sympatric); iii) somatic or vegetative incompatibility which have been used in virtual synonymy, although Esser & Kuenen (1967) distinguished the latter from heterogenic incompatibility on the basis of its restriction to the vegetative phase without influence on sexual behaviour. This distinction has been associated with confusion surrounding the description of an interaction as purely somatic, sexual or a type of combination, e.g. somatic with sexual consequences. This has been partly due to a cause/consequence dilemma and, particularly within those basidiomycetes under consideration, to the nature of primary mycelial interactions which are probably best regarded as somato-sexual (Rayner et al.,1984). It is not surprising therefore, that heterogenic incompatibility was initially appreciated within heteromictic Ascomycotina, where sexual and somatic phases are distinct, as a mechanism which reduced the smallest unit of evolution to a sub-specific category.

The system was envisaged as restricting recombination and the transfer of mutations with selective advantages (Esser, 1974) and thereby acted as an agent of sympatric speciation (Burnett, 1983). In contrast, Day (1970) and Caten (1971) suggested that the heterogenic I.S. reduced harmful cytoplasmic transfer

throughout a species by acting as a cellular defence mechanism. Indeed, evidence was soon forthcoming that transfer of "vegetative death", a degenerative cytoplasmically determined condition of Eurotium (Aspergillus) amstelodami, was reduced from 100% in heterogenically compatible combinations to 15% in those that were heterogenically incompatible (Caten, 1972). Therefore, an essential preliminary to the attempted biological control of pathogenic fungi by cytoplasmic transfer methods should be a thorough study of population structure and genetics, e.g. the spread of "Rhizoctonia decline" (Castanho & Butler, 1978; Castanho, Butler & Shepherd, 1978) and control of chestnut blight (Anagnostakis, 1983, 1984 a,b; Kuhlman & Bhattacharyya, 1984; Kuhlman et al., 1984) and Dutch elm disease (Brasier, 1983, 1984). Hartl, Dempster & Brown (1975) proposed that the heterogenic I.S. had adaptive significance by preventing heterokaryotic exploitation in N. crassa and possibly filamentous fungi in general. It was suggested that certain nuclei are non-adaptive in a homokaryon, but may exploit a heterokaryon after anastomosis by virtue of a proliferative advantage. An extreme form of such exploitation was reported in N. crassa in which the allelic pair I and i were stable in a heterokaryon, provided the proportion of I was less than 30% (Pittenger & Brawner, 1961). If the proportion of i fell below 70%, subsequent division capacity of i was decreased such that the mycelium became an I homokaryon.

Within the Basidiomycotina, heterogenic incompatibility was detected as such only relatively recently. Probably the single greatest impediment to the realization that natural populations existed as discrete units, usually secondary mycelia,

was the adoption and apparent tenacity of the unit mycelium concept which originated from Buller's (1931) work on the social organization of the Hymenomycetes. This painstaking study began with the observation of repeated anastomoses between mycelia of C. sterquilinus which grows in horse dung. Buller suggested that many small mycelia might co-operate in order to obtain sufficient nutrients from dung balls to produce basidiomata. Since this species is non-outcrossing, many or all of the neighbouring mycelia might be expected to be of identical genotype. Hence Buller's observations do not necessarily imply the existence of the extraordinary biological condition of genetic and physiological mosaicism resulting from fusion and co-operation of genetically dissimilar mycelia. Nonetheless, just such a wide ranging concept, the unit mycelium, developed from Buller's rather limited survey. Burnett & Partington (1957) analysed the genetic constitution of basidiomata of heteromictic Basidiomycotina growing in close proximity and the unit mycelium was the favoured hypothesis invoked to account for the simultaneous fruiting of secondary mycelia with overlapping mating-type factor constitutions. Raper (1968) regarded this study as a confirmation of Buller's concept in nature and further examples of the concept's persistence are provided by Raper & Flexer (1970), Lemke (1973) and Burnett (1975).

The contrasting concept that genetic dissimilarities, i.e. heterogenic incompatibility, were maintaining conspecific field isolates as discrete units in nature has increasingly gained acceptance within the last twenty years, particularly within wood-rotting species, e.g. Fomitopsis (Fomes) cajanderi (Adams & Roth, 1967), Phaeolus (Polyporus) schweinitzii (Barrett

& Uscuplic, 1971), Coriolus versicolor (Rayner & Todd, 1977), Phellinus (Poria) weirii (Hansen, 1979) and Piptoporus betulinus (Adams, Todd & Rayner, 1981). In addition, the presence or absence of an intermingling response at contact sites between conspecific basidiomata and/or superficial mycelial mats developing on transversely cut wood sections after humid incubation seemed to be under similar genetic control. This could result in an in situ expression of heterogenic incompatibility which was indicative of, and continuous with, an underlying and similarly pigmented zone within the wood (Rayner & Todd, 1982b). Examples of such relatively undecayed zones, which have been somewhat illogically termed zone lines, have been shown to contain dematiaceous hyphomycetes, e.g. Rhinocladiella spp. (Rayner, 1976). Their formation has been attributed to several biotic and abiotic factors (e.g. see Campbell, 1933, 1934, 1939; Campbell & Munson, 1936; Lopez-Real, 1975; Lopez-Real & Swift, 1975; Rayner & Todd, 1979) and this diversity may have impeded detection of the subset resulting from intraspecific heterogenic incompatibility. On semi-solid culture media, isolates from either side of this type of zone line failed to form unit mycelia and a range of incompatibility zone types has been recorded and named, e.g. killing reaction in T. cucumeris (Flentje & Stretton, 1964), interaction zone in P. schweinitzii (Barrett & Uscuplic, 1971) and others listed by Rayner & Todd (1982b). In some species the zone was not macroscopically striking, e.g. Cochliobolus heterostrophus (Ascomycotina) (Leach & Yoder, 1983). However, most studies described zones consisting variously of relatively sparse hyphae which were sometimes flanked by mounds of aerial mycelium; lysis and production of hyphal ghosts;

pigmented hyphae and/or medium and/or superficial droplets, or lack thereof between pigmented mycelia, e.g. in P. anserina.

In the Basidiomycotina, pairings between mating-type incompatible primary mycelia show a variable intensity of heterogenic incompatibility, even within a single progeny set. A common feature, however, is the subjectively assessed lesser intensity of interaction when compared with that of conspecific field isolates from different geographical sites, e.g. in S. hirsutum (Coates, Rayner & Todd, 1981) and S. gausapatum (Boddy & Rayner, 1982). These authors and others also reported a diminution of interaction intensity between laboratory-synthesized secondary mycelia associated with a corresponding increase in relatedness of their component primary mycelial genetic constitutions. Decreased interaction intensity has been associated with two particular features of the confrontation zone, i.e. diminution of pigmentation intensity (e.g. Adams & Roth, 1967; Barrett & Uscuplic, 1971; Todd & Rayner, 1978; Thompson & Rayner, 1982) and widening of the zone itself (e.g. Adams, 1982). Taken together, these results indicated that the underlying genetic basis was not simple, but probably polygenic and possibly multiallelic also (Rayner et al., 1984). This was experimentally investigated using sib-composed, sib-related secondary mycelia, i.e. all synthesized from a single progeny set of primary mycelia, of P. betulinus (Adams, 1982). This provided evidence for a single multiallelic locus (h-locus) which was implicated in the development of an aerial mycelial ridge over the confrontation zone. Surprisingly, sib-related, mating-type incompatible primary mycelial interactions did not conform to such a simple pattern. Moreover, their interactions were more

intense than those of sib-composed, sib-related secondary mycelia which led Adams (1982) to conclude that more genes for rejection were being expressed before the transition to the secondary mycelial phase.

The contrasting concepts of a heterogenic I.S. and unit mycelium raise the problem of the constitution of an individual in nature (see Pugh, 1980). An extremely unitary view is that species of fungi do not consist of discrete individuals, because the individual and species are synonymous (Kemp 1975, 1976). Several early workers recognizing manifestations of incompatibility in woody substrata referred to the delimited units variously as individuals/mycelia (Brodie, 1935), multiple infections (Roth, 1952) and clones (Childs, 1963). Hence it is clear that the concept and identification of basidiomycete individuals is beset with genetic and spatiotemporal difficulties. If we consider that a primary mycelium is an individual, the problem then arises of asexual sporulation producing mycelia that may be initially spatially defined, but genetically identical and which, in time, may intermingle, fragment and sporulate again. Heterokaryosis presents another problem, because two genetically distinct primary mycelia may be totally or only partially converted to a single secondary mycelium which, whilst maintaining its biological integrity (see Todd & Rayner, 1980; Rayner & Todd, 1982b), may nonetheless fragment, re-unite and asexually sporulate.

With regard to detecting mycelial genotypes, it is unlikely that heterogenic incompatibility, although probably polygenic, is dependent on the entire genome. Hence, when it is used as a tool to analyse population structure, the intermingling

response must not be taken to indicate genetic identity. Whereas intermingling progeny of non-outcrossing forms are probably genetically identical, intermingling mycelia derived from natural substrata may contain samples of slightly genetically dissimilar origin. Nevertheless, studies involving heterogenic incompatibility in this way provide for a much finer resolution of population structure than those which are based solely on the homogenic I.S.. Studies which are merely concerned with the latter cannot distinguish between genetically distinct secondary mycelia arising from sib mating and the parental secondary mycelium, because they will all contain the same complementary mating-type factors (see Korhonen, 1978a; Martin & Gilbertson, 1978; Ullrich & Anderson, 1978; Anderson et al., 1979; Chase & Ullrich, 1983; Kile, 1983; Stenlid, 1985).

Interplay of homogenic and heterogenic incompatibility - the concept of override

The two incompatibility systems are probably best regarded as fundamentally contrasting self/non-self recognition phenomena. The homogenic I.S. which promotes outbreeding and the heterogenic I.S. which promotes genetic isolation must frequently be brought into conflict in nature. The resulting interplay and balance between the underlying opposing tendencies of rejection and territoriality, i.e. individualism, versus acceptance and synergism, i.e. collectivism, can clearly be investigated during sexual reproduction (Rayner et al., 1984). The success or failure of mating can be envisaged as one system overruling (Carlile & Gooday, 1978), overriding (Rayner & Todd, 1979), or bypassing (Brasier, 1984) the other.

In heteromictic Ascomycotina however, the presence of sex organs seems to alleviate the conflict inasmuch as heterogenic incompatibility need not necessarily preclude outcrossing (Jinks & Grindle, 1963). Returning to P. anserina, Esser (1965) stated that each system of incompatibility existed independently of the other and that there was no interaction between the two. Nevertheless, he found that mating-type compatibility, in the presence of an inter-racial zone of somatic heterogenic incompatibility, was sometimes restricted to non-reciprocal fertilization between spermatia of one partner and trichogynes of the other and was sometimes absent altogether. This may be interpreted in terms of the homogenic I.S. being overridden to varying degrees by the heterogenic I.S. between races. Conversely, intra-racial reciprocal mating-type compatibility may be interpreted in terms of relatively small degrees of genetic dissimilarity being overridden by the homogenic I.S..

Jinks et al. (1966) showed that heterogenic incompatibility in E. nidulans was not a barrier to outcrossing by demonstrating perithecium production between a pair of heterokaryon-incompatible isolates in different h-c groups. Nevertheless, as was reported in P. anserina (Esser, 1965), the effects of heterogenic incompatibility may be expressed after fruiting, because the so-called hybrid progeny had a lower radial extension rate than either their parents or intra h-c progeny. Butcher (1968) has suggested that this, by virtue of being a fitness characteristic, could render inter h-c progeny at a selective evolutionary disadvantage.

In N. crassa, there is a dimictic homogenic I.S. and a heterogenic I.S. such that an incompatible killing reaction

follows anastomosis of mycelia carrying unlike alleles at the "het" loci (Garnjobst & Wilson, 1956). However, some aspects of the heterogenic I.S. seem dependent on units of unlike mating-type factors themselves acting as unlike "het" alleles (see Perkins & Barry, 1977). In contrast, most ascospores of Neurospora tetrasperma (and P. anserina) contain a pair of mating-type compatible nuclei and so yield heterokaryotic mycelia. That these mating-type factors also have innate "het" allele function was revealed after their introgression into the genetic background of N. crassa (Metzenberg & Ahlgren, 1973). This suggests that in such Ascomycotina, the homogenic I.S. is brought into direct conflict with the heterogenic I.S. within a single vegetative mycelium, a phenomenon which was undoubtedly fundamental to basidiomycete evolution.

In those basidiomycetes under consideration, because outcrossing occurs in the absence of sexual differentiation, the balancing, overruling, overriding or bypassing tendencies between the two incompatibility systems are crucial in the control of heterokaryosis. It seemed to Anderson (1984) that heterokaryosis which "is part of the homogenic incompatibility system is maximised when the heterogenic incompatibility system is minimised". Once a stable secondary mycelium has developed, the weight of evidence suggests that it is unlikely to form novel heterokaryons after interacting with conspecific secondary mycelia. Contrary evidence is restricted to nutritionally forced pairings, e.g. in A. bitorquis (Raper, 1976), and the equivocal results of Bolkan & Butler (1974) concerning further heterokaryosis of cultured heterokaryotic field isolates of T. cucumeris carrying different homogenic incompatibility factors

(H-factors) in pairings on a medium containing 1% charcoal. Heterokaryotic tufts of novel avirulent genotypes, i.e. mycelia containing three or four H-factors, were occasionally produced and were restricted to the narrow confrontation zones. It should be stressed that the use of charcoal in this connection was reported as being essential for tuft formation and so may have contributed to the forced override of the heterogenic I.S. by the presence of different mating-type factors. This is supported by Elliott's (1978) work on A. macrosporus in which the addition of 0.5% w/v activated charcoal increased the number of putatively mating-type compatible sib interactions which actually satisfied his criteria of successful mating.

Circumstantial evidence for a fine balance existing between acceptance and rejection of non-self was provided by the confrontation zones of paired mating-type compatible primary mycelia of T. cucumeris (e.g. Anderson et al., 1972). These zones contained manifestations of both the killing reaction and heterokaryosis with tuft formation. A similar dual acceptance/rejection expression may also underlie the production of a clear zone, ("barrage") about 1-2mm wide, free of aerial hyphae and of reduced hyphal density which delayed the emergence of secondary mycelium in certain mating-type compatible pairings of Polyporus brumalis and Polyporus ciliatus (Esser & Hoffmann, 1977; Hoffmann & Esser, 1978). An analysis of the genetic determinants controlling this reaction in P. ciliatus, which involved three inbreeding cycles, revealed the interaction of three independent genes "in a way characteristic for systems of heterogenic incompatibility", (Hoffmann & Esser, 1978). However, such a phenomenon seems to be due to delayed anastomosis rather

than a manifestation of heterogenic incompatibility in its usual post-fusion context. Nevertheless, although the authors did not correlate the two events, gradual hyphal lysis began to occur in the "barrage" region 2-4 weeks after mycelial contact.

The preceding observations have been incorporated into a suggestion which may be generally applicable within the Basidiomycotina, i.e. in order for outcrossing to occur, some mechanism is necessary to override any heterogenic incompatibility between different homokaryons until secondary mycelial establishment. Thereafter, the heterogenic I.S. operates to prevent further nuclear entry and migration (Rayner & Todd, 1979; Rayner & Turton, 1982). Several exceptions, where the heterogenic I.S. has apparently overridden the homogenic I.S., i.e. non-mating of otherwise outcrossing sibs of unlike mating-type, have been reported in British S. hirsutum (Coates et al., 1981) and S. gausapatum (Boddy & Rayner, 1982). Interestingly in S. hirsutum, inbreeding, which would be expected to reduce genetic difference, resulted in progeny whose complementary mating-type interactions showed full mating competence similar to those of non-sibs derived directly from field isolates (Rayner et al., 1984).

This led to a graphic representation of possible fluctuations in acceptance and rejection with genetic differences in S. hirsutum (Fig. 1.1). Different patterns, perhaps merely extrapolations and interpolations of the curves shown in Fig. 1.1, seem to be emerging for other Basidiomycotina, e.g. pairings of T. cucumeris primary mycelia from field isolates of widely separated geographical origin tend to tip the acceptance/rejection balance towards an overriding rejection

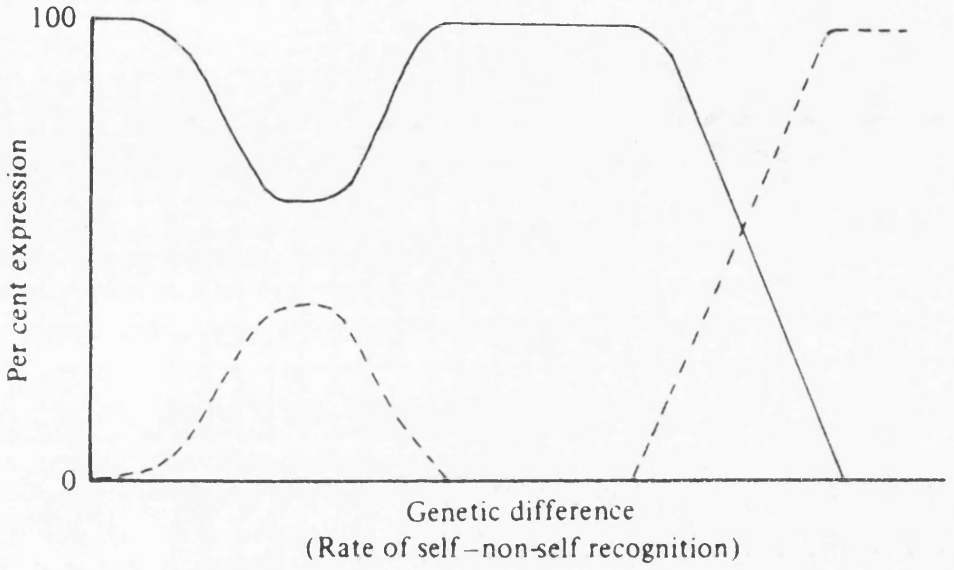


Fig. 1.1. Possible fluctuation in expression of rejection and acceptance in mating-type compatible primary mycelial interactions in S. hirsutum associated with genetic difference. Continuous line, acceptance; dashed line, rejection (after Rayner et al., 1984).

(Anderson, 1984). In Athelia (Sclerotium) rolfsii, a sample of 50 homokaryons showed ca. 80-90% rejection in both sib and non-sib pairings (Punja & Grogan, 1983), suggesting that the level of acceptance detected in outcrossing Stereum spp. might only be reached or approached in A. rolfsii after several cycles of inbreeding.

Such fluctuations may also underlie the results of Fatemi & Nelson (1978) who reported an interesting lack of heterogenic incompatibility between isolates of Pyricularia oryzae (Ascomycotina) derived from widely separated geographical areas. They concluded that on an agar medium containing polished rice plus Rose Bengal, monoconidial cultures (homokaryons) derived from different field isolates sometimes produced heterokaryons with greater frequency than those derived from the same field isolate. Applying the override hypothesis, the degree of genetic dissimilarity required for an overriding rejection response might not have been reached between interacting homokaryons from different field isolates. Further examples of override putatively occurring in ascomycete interactions are given on pages 53-54.

Another important example of the two incompatibility systems tending to operate together in space and time, thus posing "the paradox of simultaneously promoting and restricting genetic exchange" (Day, 1978), is the Buller Phenomenon. This involves a conspecific secondary/primary mycelial interaction in which one or both mating-types contained within the secondary mycelium is/are mating-type compatible with that of the primary mycelium. Following anastomosis, nuclei which satisfy this condition may migrate from the secondary to the primary mycelium.

When such interactions involve three compatible mating-types, the primary mycelium may be superseded by heterogenically incompatible secondary mycelia of two different composite genotypes. Such secondary mycelial interactions within a single ex-primary mycelium may be visible as "tracks" delimiting either mycelial sectors, as was reported in C. versicolor (Todd & Rayner, 1978; Rayner & Todd, 1979), or strips, as in S. hirsutum (Coates et al., 1981). In the latter, heterogenic incompatibility was often evident between inoculated secondary and primary mycelial types before detection of composite secondary mycelia. This may correspond to the relative delay times between initiation and detection of heterogenic incompatibility and nuclear migration, and perhaps also reflects a shift over time in the ratio of rates of initiation of the two processes.

The possibility of a heterokaryon's nuclear components competing for mating-type compatible nuclei within a homokaryon, resulting in preferential selection of one or other composite heterokaryon, has been the subject of much investigation although the mechanism remains unresolved. Kimura (1958) used strains derived from wild basidiomata of bifactorial Coprinus macrorhizus f. microsporus and Psilocybe coprophila. He found that when one nuclear type within a heterokaryon was sib-related to the homokaryon's nuclei, the composite heterokaryon was preferentially synthesized from the more genetically unrelated, and hence presumably less similar, nuclear combination. Non-sib selection was interpreted in terms of at least one series of multiple alleles which modified the effects of the homogenic incompatibility factors. Crowe (1963) approached the problem in S. commune using highly isogenized laboratory strains, but, apart

from allowing her to implicate a role for the incompatibility factors themselves, the preparatory inbreeding regime, and hence greatly reduced heterogenic I.S., did not seem to be helpful. The important relationship between heterogenic incompatibility and non-sib selection has recently been investigated in S. hirsutum by Coates, Rayner & Boddy (1985). They found that the passage of nuclei bearing a sib relationship to those of a recipient homokaryon from a heterokaryon through an intervening zone of pre-formed or developing heterogenic incompatibility was effectively delayed relative to that of non-sib-related nuclei. A maximum delay time of 127.4h at 25°C was recorded for a pre-formed zone which was based on a comparison of the areas occupied by the composite heterokaryons.

There are many documented cases in which the override mechanism, operating in the original sense of allowing outcrossing within the Basidiomycotina, seems to be inoperative. Such a failure may result in heterogenic incompatibility barriers which delimit intersterile outcrossing units, i.e. biological species or hologamodemes in the terminology originally proposed by Gilmour & Gregor (1939). Such barriers may occur between allopatric populations, or within local breeding populations (gamodemes) and act as agents of sympatric isolation. Clearly this poses further problems relating to consequence and cause of accrued genetic differences, heterogenic incompatibility barriers and sexual reproduction.

Macrae (1967) recognized a species within the Hirschioporus (Polyporus) abietinus aggregate which had a poroid hymenium and consisted of two intersterile North American groups (A,B) which were partially interfertile with a third group (C)

from Europe. Such a relationship has been repeatedly demonstrated in other species and is now generally described as the A-B-C scheme, e.g. in Coprinus bisporus (Kemp, 1980b) and three species of Amylostereum (Boidin & Lanquetin, 1984a). Such schemes indicate the need for caution before assigning two intersterile breeding units to different biological species.

Further examples of reproductive isolation in which the homogenic I.S. putatively fails to override the heterogenic I.S. include the bifactorial A. mellea complex, which also contains an A-B-C relationship between two intersterile North American "biological species" and a European one, (e.g. Korhonen, 1978a; Ullrich & Anderson, 1978; Anderson, Korhonen & Ullrich, 1980) and the nomenclatural entity Sistotrema brinkmannii. The latter comprises "unifactorial, bifactorial and homothallic" forms with intersterile subsets within the former categories (Ullrich, 1973). However, the interplay of homogenic and heterogenic I.S. within this complex has only been investigated using biochemically forced crosses of auxotrophic mutant primary mycelia (e.g. Lemke, 1969; Ullrich & Raper, 1975). Such crosses between bifactorial and other forms failed to reveal any genetic recombination, however such a degree of isolation was not present between the non-outcrossing and unifactorial forms and within the former. The presence of at least two breeding strategies within a species, or morphologically similar species, has also been documented in Heterobasidion annosum (Korhonen, 1978b; Chase & Ullrich, 1983; Chase, Ullrich & Korhonen, 1985), other Eumycota (e.g. Burnett, 1965, 1975, 1983) and in the Myxomycota (e.g. Collins, 1979; Collins et al., 1983; Collins & Gong, 1985).

Notwithstanding the possible differences in cytological behaviour in basidia of non-outcrossing forms, e.g. karyogamy and genetic recombination between auxotrophic isolates of S. brinkmannii (Lemke, 1969), and rare karyogamy in S. sanguinolentum (Robak, 1942), the consequences for natural population structure will be the same (Rayner & Turton, 1982). The resultant closely or clonally related heterogenically incompatible field isolates may be taxonomically recognized as microspecies or aggregate complexes and their interactional behaviour has been expressed by Gregory (1984) as follows, "membership of the co-op is restricted to members of the clone". With the exception of secondary mycelial establishment, this statement succinctly defines the currently accepted limits of Buller's unit mycelium concept.

NUCLEAR BEHAVIOUR - THE HOLOCOENOCYTIC CONDITION

Boidin (1971a) recognized five categories of nuclear behaviour in the Basidiomycotina and arranged them in order of increasing predominance of the multinucleate state in the life cycle. Normal behaviour, the commonest condition within the Aphyllophorales, describes a life cycle with uninucleate basidiospores and primary mycelial compartments. Binucleate compartments are produced during the secondary phase and reversion to the uninucleate condition occurs after karyogamy within basidia. A primary mycelium with uninucleate compartments is by definition a monokaryon and if the nuclei are of a single genotype it is a homokaryotic monokaryon, although this is often contracted to monokaryon. Similarly, binucleate compartments in

a secondary mycelium render it a heterokaryotic dikaryon, but in this case the contraction to dikaryon can often lead to confusion. A range of nuclear conditions, their nomenclature and abbreviations is provided by Burnett (1976).

Holocoenocytic nuclear behaviour was placed at the opposite end of the range to that described as normal because the multinucleate condition is maintained throughout the mycelial phases and retained in the basidioma "sometimes even up to the base of basidia", e.g. C. puteana (C. cerebella) (Boidin & Lanquetin, 1984b). The basidiospores are either uninucleate or, following a post-meiotic mitosis, binucleate. Thus the terms monokaryon and dikaryon are inapplicable and hence the interactions of the Buller Phenomenon have to be described, not as di-mon matings, but as secondary/primary or heterokaryon/homokaryon matings.

Boidin and coworkers have routinely used the detection of clamp connections in the determination of sexual behaviour and its correlation with nuclear condition (e.g. Boidin, Terra & Lanquetin, 1968; Boidin, 1971a; Boidin, 1980). When considering non-fruiting clampless species which have binucleate or multinucleate primary mycelial compartments and morphologically indistinguishable cultures of monosporous and polysporous origin, Boidin (1971a) has judged them to be "doubtfully homothallic", e.g. Hymenochaete tabacina, or presumably homothallic, e.g. Peniophora reidii (Boidin & Lanquetin, 1983). Furthermore, he recognized the possibility of a concealed haploid parthenogenetic (asexual) system and qualified his application of the term homothallic by stating "it is not within the true definition of this term" (Boidin, 1971a). However, since Boidin (1958) did not

observe any morphological differences between monosporous and polysporous cultures of holocoenocytic Basidiomycotina, their apparently less dubious homothallism seems to be based on the presence of clamp connections. These may occur in pairs or whorls (verticillate) and even on primary mycelia in some species. In view of the historical allocation of weight to the scoring of clamp connections, this probably led to the "dominant correlation" stated by Boidin (1971a), i.e. "holocoenocytic behaviour, homothallic, verticillate clamps, spores binucleate" and the statement "the genus Stereum s. st. is very difficult to delimit specifically, because they are all fixed homothallic lines and there is no crossing between them" (Boidin, 1971b). Such statements seem to have fostered a general belief that holocoenocytic Basidiomycotina are invariably homothallic (e.g. Eriksson, Hjortstam & Ryvarden, 1978, 1984; Jülich & Stalpers, 1980; Ginns, 1982; Hallenberg & Eriksson, 1985). Boidin's group however, have recently revived the caution and hesitation expressed by earlier workers on such fungi over the use and meaning of the term homothallic (e.g. Kniep, 1928; cited by Boidin & Lanquetin, 1984b; Kemper, 1937; Herrick, 1939; Robak, 1942).

A WORKING HYPOTHESIS ARISING FROM RECENT STUDIES OF MYCELIAL INTERACTIONS IN Stereum spp.

Despite the previously assumed homothallism, evidence for the existence of outcrossing British members of the genus Stereum has been accumulating since the beginning of the 1980s. The production of a distinct secondary mycelium on both morphological

and interactive criteria has been reported in S. hirsutum (Coates et al., 1981), S. gausapatum (Boddy & Rayner, 1982) and S. rugosum (Rayner & Turton, 1982). Sib matings revealed that all three species contained forms with a unifactorial homogenic I.S. and non-sib matings revealed a diaphoromictic breeding strategy. Nevertheless, the pattern of secondary mycelial establishment was specific to each species as indicated below.

In S. hirsutum, secondary mycelium developed throughout the mated primary precursors producing a more or less uniform aerial morphology across the culture dish (Coates et al., 1981). One type of mating-type incompatible sib interaction, bow-tie, involved the development of a band of appressed and sparse mycelium, widest near the ends of the confrontation zone and bounded by narrow regions of exuded watery droplets. Hyphae within this band had widely separated regions of very dense branching which resembled the "flat" and "blotchy" hemicompatible-A morphology of bifactorial forms such as S. commune. Furthermore, mating and bow-tie production have each been accommodated within single multiallelic locus models (Coates et al., 1981; Coates & Rayner, 1985a), thus conferring some aspects of the bifactorial system on the essentially unifactorial S. hirsutum. Bow-ties encroached unilaterally or bilaterally into the resident primary mycelia and ultimately persisted or regenerated one or both original primary mycelial types. A zone of heterogenic incompatibility was usually produced at the interface of regenerating mycelia, or between one such mycelium and the remaining portion of an inoculated type, which did not always coincide with the original confrontation zone, i.e. partial mycelial replacement could occur.

A reaction resembling the bow-tie occurred in S. gausapatum, but the unilaterally or bilaterally expanding zone of appression (lytic crescent) was initially widest at its midpoint, could occur between non-sibs and sometimes preceded secondary mycelial emergence (Boddy & Rayner, 1982). In contrast, no such reaction was observed in S. rugosum (Rayner & Turton, 1982). Instead, secondary mycelial establishment was sometimes preceded by a narrow, truly lytic reaction at the confrontation zone which contained sparse and dead hyphae. In order to try to accommodate this range of interactions within a single framework, Rayner et al. (1984) proposed a working hypothesis which distinguished three separate processes operating in various combinations during secondary mycelial development as listed below.

Access migration

This refers to a type of nuclear migration whose rate is dependent on the genotypes of both donor and recipient isolates. Operating alone, access results in appression of aerial mycelium, inhibition of colony margin extension and production of hyphae with sparsely distributed regions of dense branch proliferation, as occurs in the bow-tie reaction of S. hirsutum. The characteristic shape may develop because of impeded nuclear migration radially towards the older centre of a recipient mycelium. This possibility has received scant attention since Buller's (1931) experiments on Coprinus radiatus (C. lagopus sensu Buller, C. fimetarius sensu Bensaude) in which all of the centre or parts of the periphery were removed from a homokaryotic mycelium before a mating-type compatible homokaryon

was inoculated at its margin. He determined the time elapsing before distinct secondary mycelium was visible at that point on the resident colony margin which was furthest from the mating-type compatible inoculum. These and related experiments led Buller (1931) to conclude that the periphery of a mated homokaryon may contain secondary mycelium whilst its central portion remained in the primary phase. Furthermore, rather than moving radially through the central portion of a mycelium, migratory nuclei seemed to move preferentially via the younger peripheral portions, i.e. circumferentially, where no throughgrowth of invading hyphae was obvious (Buller, 1931; Swiezynski & Day, 1960). Such a pattern of nuclear migration, coupled with the possible slower circumferential movement of donor nuclei by repeated anastomosis between heterokaryotic and homokaryotic hyphal compartments, has been advanced to account for the expansion of the bow-tie region in S. hirsutum (Coates & Rayner, 1985a).

Production of a hyaline confrontation zone in mating-type compatible pairings of S. rugosum is probably due to an expression of heterogenic incompatibility which affects secondary phase establishment by preventing a proportion of anastomoses from initiating nuclear migration. This lytic region did not expand into the neighbouring residents, nor did it contain the characteristic morphological attributes of a bow-tie reaction. However Rayner & Turton (1982) detected mottled regions of appressed mycelium within the central region of certain paired mating-type compatible primary mycelia and so unstabilized access migration cannot be discounted in this species.

Acceptor migration

In their study of the interactive behaviour of mycelia developing from plug inocula removed from mating-type compatible interactions in S. hirsutum, Coates et al. (1985) concluded that there were no significant differences ($P>0.05$) in nuclear migration rates between different donor nuclei following mycelial implantation into a recipient isolate. Although access migration was interpreted as an initial phase of the mating process, and hence analogous to the morphological expression of B-on which precedes that of A-on in S. commune (Raper, 1983), it seemed to be superseded by a fundamentally different mechanism. Hence the term acceptor migration was proposed for those situations in which nuclear migration had a greater radial component, was apparently faster and occurred at less variable rates than had been detected in bow-tie expansion during access migration in S. hirsutum.

Stabilization

This results in a stable heterokaryotic association after access and acceptor migration, or access migration acting alone, which can then propagate itself as a balanced fertile secondary mycelium. If stabilization fails to occur, the result may be a "flat" or "blotchy" common mating-type factor heterokaryon which may revert to one or both component homokaryons. In the example of S. hirsutum, acceptor migration is stabilized, hence secondary mycelium emerges from subcultures taken from any point within mating-type compatible pairings after a sufficient period of incubation. If access migration was stabilized instead, then secondary mycelium would be expected to develop in a bow-tie shape surrounded by resident primary mycelial remnants as has

been reported in Mycena galopus (Frankland, 1984). Indeed, subcultures removed from slowly and bilaterally expanding or persistent bow-ties of S. hirsutum rarely produced a woolly-floccose mycelium resembling that of the secondary phase (Coates & Rayner, 1985a). In such cases, it seems that mating-type incompatible heterokaryons were stabilized solely by the action of dissimilar bow-tie factors. If stabilized access migration preceded stabilized acceptor migration, then initial bow-tie shaped regions of secondary mycelium would be expected to be obliterated by subsequent secondary mycelial establishment within adjacent regions of the resident primary mycelia.

In S. gausapatum, stabilized acceptor migration within mating-type compatible combinations seems to occur either rapidly, or following a more prolonged period of access migration. The latter results in appression zones (lytic crescents) which, on the basis of morphology and interactions after subculturing, contain secondary mycelium (see Boddy & Rayner, 1982).

Applicability of the working hypothesis within the Coprinaceae

Kemp's (1980a) new system of homogenic incompatibility within the Coprinaceae (see p. 26) is based on secondary mycelial establishment occurring either after, or without, nuclear migration. If such mycelium failed to emerge from either of two plugs removed from positions 1cm on either side of the confrontation zone in a 7-9 day old pairing, he concluded that nuclear migration was not occurring. However, since this study did not involve direct microscopic observations of living hyphae, it did not completely discount the possibility of narrow bow-tie shaped regions of stabilized, but slow, access migration which

could equally apply to the initial stages of slow nuclear penetration in C. disseminatus (Butler, 1972). Hence the sib mating patterns in forms with the new system conform to a unifactorial scheme, but mating-type compatibility per se masks the action of two incompatibility factors. One of these can be interpreted as regulating stabilized access migration (fertility factor sensu Kemp) which in C. disseminatus is possibly followed by stabilized acceptor migration, whilst both acting in concert (migration and fertility factors sensu Kemp) regulate stabilized acceptor migration in which any initial access component is not macroscopically expressed. Since the two interpretations basically differ in the presence or absence of nuclear migration within the confrontation zone, direct examination of post-fusion events in living hyphae could be a useful test.

Applicability of the working hypothesis within the Ascomycotina

This working hypothesis may be applicable within the Ascomycotina and help to elucidate some fundamentally similar processes operating throughout the higher fungi. Morphologically distinct, bow-tie shaped regions, possibly resulting from override and access migration have been observed between pairs of monosporous and stromatal isolates within the genera Daldinia and Hypoxylon (Rayner et al., 1984; Sharland & Rayner, 1986). In these cases, heterokaryotic stabilization was rarely observed following hyphal tip isolation, instead putative heterokaryons usually reverted to one or other or both primary mycelial components.

There are also similarities with the d-reaction and penetration effect in Ophiostoma (Ceratocystis) ulmi. However, in the former, Brasier (1983, 1984) found evidence for the

exchange of cytoplasmic factors only, while in the latter, he interpreted widening interaction zones not as indications of access phenomena, but of full heterogenic incompatibility associated with the likelihood of mycelial introgression (see pp. 108-109).

Aims of the project

The investigation of incompatibility systems within a diverse range of fungi is an essential prerequisite for the formulation of general principles and selection of model systems for detailed study. Watling (1971) has drawn attention to the fact that only a small proportion of higher fungi has been subjected to intensive genetic studies. Indeed, by far the most frequently studied basidiomycetes in this respect have been S. commune (e.g. Raper, 1966a; Raper, 1983) and Coprinus cinereus (e.g. Casselton & Economou, 1985), both of which have uninucleate primary mycelial compartments, regularly clamped binucleate secondary mycelial compartments and bifactorial diaphoromictic homogenic I.S.

The study of fungal incompatibility systems may facilitate investigations of analogous phenomena, for example angiosperm sexuality (e.g. Lewis, 1954, 1979; Frankel & Galun, 1977; Pandey, 1977), invertebrate graft rejection (e.g. Theodor, 1970; Neigel & Avise, 1983a,b) and vertebrate immunological processes (e.g. Burnet, 1971). Furthermore, it permits the integration and critical assessment of fungal self/non-self recognition within the framework of microbial systems (e.g. Van den Ende, 1976; Crandall, 1977; Curtis, 1978; Lane, 1981; O'Day &

Horgen, 1981), plant physiology (e.g. Linskens & Heslop-Harrison, 1984) and the life sciences as a whole (e.g. Monroy & Rosati, 1979).

The holocoenocytic Basidiomycotina are particularly interesting organisms for research in this field, not only for their extended multinucleate phase and its biological implications, but also for the economic importance of some species in the decay of standing and processed timber. Evidence for outcrossing within Stereum (see pp. 47-52) has led to a re-examination of interfertility criteria, support for the override and individualistic mycelium concepts and the possibility of detecting breeding units and barriers within holocoenocytic Basidiomycotina as has been achieved for example in Serpula (Merulius) (Harmsen, 1960), Coprinus (e.g. Kemp, 1970, 1975), Coriolus (Edwards & Kennedy, 1973), and more recently in Armillaria (e.g. Anderson et al., 1980), Collybia (Vilgalys & Miller, 1983), Laccaria (Fries & Mueller, 1984), Thanatephorus (e.g. Anderson, 1984) and Agaricus (Anderson et al., 1984). The importance of interfertility testing was realized by Nelson (1963) who regarded it as a direct measure of relationship and important in measuring the significance of the description of a fungal species. Certainly Stereum is in need of such an experimental approach since it contains a plethora of synonyms and aggregations of taxa in species complexes (e.g. Lentz, 1955; Welden, 1971).

In view of the abundant confusion and assumptions associated with the genus, the mycelial interactions of the lesser-studied or neglected members were investigated from various standpoints including incompatibility, genetics,

development, ecology and population biology. Comparable work within Phanerochaete and Coniophora is non-existent and so the intraspecific interactions of P. laevis, P. velutina and C. puteana were also investigated to extract the themes and variations associated with the holocoenocytic condition. Cultured material of P. velutina proved so favourable for direct observation of living hyphae that it was chosen for a complementary cytological study of hyphal interactions and forms Part II of the project.

CHAPTER 2 ROUTINE MATERIALS AND METHODS

COLLECTION AND ISOLATION PROCEDURE

Slivers of decaying wood bearing single or confluent fresh basidiomata of British wild holocoenocytic Basidiomycotina were removed and each item immediately placed, hymenium downwards, on cellophane film in separate 9cm diameter Petri dishes to minimize contamination of spore prints. Following overnight storage at 15°C or ambient temperature, a fragment (ca. $4mm^2$) of each basidiospore-coated film was agitated in ca. 2ml sterile distilled water and the spore suspension was spread over the surface of four 9cm diameter Petri dishes, each containing 2% MAN (see Appendix i). Adjustments were made by the dilution or addition of spore suspension to facilitate future germling isolation and the dishes were incubated at 20°C in darkness, either overnight or until germination occurred. This temperature and lighting regime was followed for the incubation of all culture dishes used in this project which contained 2% MA (see Appendix i) as a medium component. All dishes used were of 9cm diameter and non-vented unless stated otherwise.

Spores of Stereum spp. usually germinated within 24h, those of Phanerochaete spp. germinated over a period of 4-5 days beginning ca. 3 days after sowing and those of C. puteana also began to germinate over several days, but after a delay of ca. 3 weeks. This delay was reduced to ca. 6 days by inoculating a conspecific primary mycelium at the edge of the seeded dishes. Germination was initially proximal to this mycelium and subsequently occurred at increasing distances from its point of inoculation.

Single basidiospore (monosporous) isolates were obtained by marking (Keyworth, 1959) and subsequent transfer of solitary unbranched germlings to 2% MA plates using a sterile tungsten needle sharpened in molten sodium nitrite. Isolation was sometimes achieved entirely on 2% MA and it was concluded that subsequent cultural behaviour was not affected by the antibiotic novobiocin.

When basidioma thickness permitted, tissue isolates were obtained from the interior using flame-sterilized tools to transfer scrapings to Petri dishes containing 2% MANB (see Appendix i). Thinner basidiomata necessitated the regeneration of mycelium from splinters of adjacent wood (ca. $2mm^3$). Care was taken to reduce the chance of isolating neighbouring mycelia by avoiding interaction zones. Whenever possible, isolates were obtained directly from dishes containing 2% MA only. By comparison it was concluded that Benomyl-assisted isolation did not alter cultural behaviour.

BASIDIOMA AND ISOLATE CODES

Collected basidiomata and material received by post were given a site or sender code consisting of an upper case letter(s) followed by a code number. Field isolates derived directly from them, or indirectly from adjacent decayed wood were distinguished by the lower case letters t and w respectively. Each monosporous isolate was numbered and this was separated from the basidioma code by a hyphen, e.g. F5t refers to a tissue isolate from basidioma 5 collected at site F and F5-15 refers to monosporous isolate 15 derived from the same basidioma. Collection sites and

isolate codes are listed in Tables 2.1-2.10. However, for convenience, the monosporous isolate code numbers therein refer to the total number of sibs isolated from a basidioma, e.g. F5-15 indicates that consecutively numbered sibs F5-1 to F5-15 were obtained from F5. Codes followed by the lower case letters a or b refer to the collection of two basidiomata which were either touching but non-confluent, or in close proximity on the same substratum.

Isolate codes may also be combined to denote laboratory-synthesized secondary mycelia, thus F25-13/U5-6 was subcultured from a pairing of two mating-type compatible, non-sib-related primary mycelia originating from two different sites, whereas F25 14/16 refers to a sib-composed secondary mycelial isolate.

USE OF THE NAME S. "rameale"

It seems that the name S. rameale cannot be applied to those collections listed in Table 2.1.. The name itself is confusing because American authors (e.g. Lentz, 1955; Chamuris, 1985a) have used it in synonymy with S. complicatum which does not occur in Britain. European authors (e.g. Jahn, 1971; Jülich & Stalpers, 1980) however, have used it, albeit with a different authority, in synonymy with S. ochraceo-flavum and S. sulphuratum. D. Reid (pers. comm.) and J. Ginns (pers. comm.) are both of the opinion that the British material studied in this project fails to conform to the original descriptions of either S. ochraceo-flavum or S. sulphuratum, principally in the absence of a yellow hymenium. Since this situation has not been resolved, the name S. "rameale" has been adopted throughout.

Table 2.1. Collection sites and isolate codes for S. "rameale" (see p. 59).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
9.x.81	Detached oak (Quercus sp.) branch, Friary Wood, Avon	ST783592	Y1w, Y1-15
18.viii.82	As preceding entry	ST785588	Y2w, Y2-5
13.x.82	As preceding entry	ST787588	Y11w, Y11-5
27.x.82	As preceding entry	ST784592 *	Y12w, Y12-5
12.x.83	As preceding entry	ST784593 *	Y13wa,b, Y13-5a,b
18.ix.82	Detached oak (Quercus sp.) branch, Asham Wood, nr. Frome, Somerset	ST707457 *	AW1w, AW1-5
31.x.82	Fallen birch (Betula sp.) trunk, Arger Fen, Suffolk	TL933355 **	AR1w, AR1-5
22.xi.82	Detached cherry-laurel (Prunus laurocerasus) branch, Farnham, Surrey	SU830452 *	FA1-6

Table 2.1. (Continued).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
18.xii.82	Detached oak (*Quercus* sp.) branch, Chepstow Park Wood, Gwent, Wales	ST484974	CP5-5
4.1.83	Dead blackthorn (*Prunus spinosa*), Tregunna, Cornwall	SW966739	TG1-5
13.ii.83	Dead attached oak (*Quercus* sp.) branch, Lord's Wood, nr. Pensford, Avon	ST634632	L2-5
6.iii.83	Detached cherry-laurel (*P. laurocerasus*) branch, Burwalls, Avon Gorge, Avon	ST563729	SB1-5
6.iii.83	Dead attached oak (*Quercus* sp.) branch, Stokeleigh Camp, Leigh Woods, Avon	ST557734	ST1-5
9.x.83	Detached oak (*Quercus* sp.) branch, nr. Stokeleigh Camp, Leigh Woods, Avon	ST555736	ST2-5
27.iii.83	Fallen oak (*Quercus* sp.),Lennox Farm Plantation, nr. Longridge, Lancs	SD654395	PF4w, PF4-5
2.x.83	Dead attached cherry-laurel (*P. laurocerasus*) branch, Hermitage Wood, nr. Purdown, Bristol, Avon	ST617774	PU1-5

Table 2.1. (Continued).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
12.x.83	Detached oak (*Quercus* sp.) branch, Wetmoor, nr. Wickwar, Avon	ST744878 *	WT1-5
31.x.83	Detached oak (*Quercus* sp.) branch, Farleigh Wood, Somerset	ST790562	F29-5
7.xi.83	As preceding entry	ST795560 **	F28-5

Isolates obtained from fresh material kindly supplied by *A.D.M. Rayner and **R.C. Dryden

Table 2.2. Collection sites and isolate codes for S. sanguinolentum.

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
3.x.78	Fallen spruce (Picea abies) trunk, Vantaa, Finland	*	K1-6 (781003.1.9)
6.x.78	Fallen spruce (P. abies) trunk, Tammela, Finland	*	K2-7 (781006.4.2)
24.x.78	Spruce (P. abies) stump, Jokioinen, Finland	*	K3-5 (781024.1.8)
9.x.80	Spruce (P. abies) stump, Loppi, Finland	*	K4t, K4-8 (801009.2.1)
16.x.80	Pine (Pinus contorta), Eurajoki, Finland	*	K5t, K5-8 (801016.1.1)
12.x.81	Fallen larch (Larix sp.) trunk, nr. deer enclosure, Ashton Park, Avon	ST551720	A1-15
8.viii.82	Fallen pine (Pinus sp.) trunk, site as for preceding entry	ST550720	A2-3
12.ii.83	Fallen pine (Pinus sp.) trunk, Beggar Bush Lane, Ashton Park, Avon	ST548726	A3-3
12.ii.83	Fallen larch (Larix sp.) trunk, site as for preceding entry	ST548726	A4-3

Table 2.2. (Continued).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
29.v.83	Detached pine (Pinus sylvestris) branches and fallen trunks, Quarry Wood, Ashton Park, Avon	ST540713	A5-3, A6-3, A7-3, A9-3, A10-3, A11-3
29.v.83	Detached larch (Larix sp.) branch, site as for preceding entry	ST540713	A8-3
22.xi.81	Detached larch (Larix sp.) branches, Rownham Hill Wood, nr. Ashton Park, Avon	ST556725	RH1-5, RH2-5, RH3-5
22.xi.81	Detached spruce (P. abies) branch, site as for preceding entry	ST556725	RH4-5
16.xi.81	Detached larch (Larix sp.) branches, Swangrove Wood, Avon	ST79563	SW1-5a,b, SW2w, SW2-5, SW3-5, SW4-5, SW6-5 SW7-5, SW8-5a,b SW9-5, SW10-5, SW12-5, SW13-5, SW14-5, SW15-5, SW16-5, SW17-5, SW19-5, SW20-5, SW21-5, SW22-5, SW24-5, SW25-5

Table 2.2. (Continued).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
31.i.82	Detached larch (*Larix* sp.) branch, Forest of Dean, Gloucs.	SO602087	D1-5
31.i.82	Detached pine (*Pinus* sp.) branch, site as for preceding entry	SO602087	D2-5
7.x.82	Detached spruce (*Picea* sp.) branches and fallen trunks, Serridge Inclosure, Forest of Dean, Gloucs	SO614145	D3-3, D4w, D4-3, D6w, D6-3, D7w, D7-3, D8w, D8-3, D9w, D9-3, D10w, D10-3, D11wa,b, D11-3a,b
7.x.82	Detached pine (*Pinus* sp.) branch, site as for previous entry	SO614145	D5w, D5-3
14.iii.82	A pile of larch (*Larix* sp.) poles, Clifford Farm, River Teign valley, Devon	SX783896	RT1-5, RT2-5, RT3-5, RT4-5, RT5w, RT5-5, RT6-5, RT7-5, RT8w, RT8-5, RT9w, RT9-5, RT10w, RT10-5
9.vii.82	Detached larch (*Larix* sp.) branches, Stoke Woods, Devon	SX932964	SK1w, SK1-3, SK2w, SK2-3, SK3w, SK3-3, SK4w, SK4-3

Table 2.2. (Continued).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
18.viii.82	Detached larch (*Larix* sp.) branches, Friary Wood Avon	ST785591	Y6-3, Y7-3
13.x.82	Detached pine (*Pinus* sp.) branch, site as for preceding entry	ST783591	Y9-3
27.viii.82	Detached spruce (*Picea sitchensis*) branches, Beacon Fell, Lancs	SD574428	BF1-3, BF2-3
29.viii.82	Fallen larch (*Larix* sp.) trunk, Goblin Combe, Avon	ST482648	GC1-3
4.ix.82	Detached pine (*Pinus* sp.) branch, Towerhead Wood, Avon	ST407593	S1-3
30.x.82	Detached pine (*Pinus* sp.) branches, Rendlesham Forest, Suffolk	TM323506 **	R1w, R1-3, R2w, R2-3
6.xii.82	Detached larch (*Larix* sp.) branches, Westonbirt Arboretum, Gloucs	ST857903	WA1w, WA1-3, WA2w, WA2-3

Table 2.2. (Continued).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
18.xii.82	Fallen larch (*Larix* sp.) trunk, Chepstow Park Wood, Gwent, Wales	ST484974	CP1-3
18.xii.82	Fallen spruce (*Picea* sp.) trunks, site as for preceding entry	ST484974	CP2-3, CP3-3, CP4-3
28.xii.82	Detached pine (*Pinus* sp.) branches, Rivington Moor, nr. Horwich, Gtr. Manchester	SD676123	HM1-3, HM2-3, HM3-3
28.xii.82	Detached spruce (*Picea* sp.) branch, site as for preceding entry	SD676123	HM4-3
26.i.83	Coniferous gate post, University of Bath, Claverton Down, Avon	ST777643 **	U3-3
26.i.83	Fallen coniferous trunk, site as for preceding entry	ST777643	U4-3

* Finnish isolates kindly supplied by K. Korhonen; original codes in parentheses.
** Isolates obtained from fresh material kindly supplied by R. C. Dryden.

Table 2.2. (Continued).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
13.ii.83	Detached pine (*Pinus* sp.) branch, Lord's Wood, nr. Pensford, Avon	ST634632	L1-3
27.iii.83	Detached pine (*Pinus* sp.) branches, Lennox Farm Plantation, nr. Longridge, Lancs	SD654395	PF1-3, PF2-3, PF3-3
20.v.83	Detached pine (*Pinus* sp.) branches, University of Bath, Claverton Down, Avon	ST777643	U6-3, U7-3a,b
31.x.83	Felled Douglas fir *Pseudotsuga menziesii*, Farleigh Wood, Somerset	ST792561	F30-3

Table 2.3. Collection sites and isolate codes for S. subtomentosum.

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
2.x.81	Fallen beech (Fagus sylvatica) trunk, Farleigh Wood, Somerset	ST792560	F5t, F5-15
4.xi.82	?, Epping, Essex	*	E1-15
8.iii.83	Fallen tree, Ledlington, Beds	*	KW1-5
9.xi.83	Fallen hazel (Corylus avellana) trunk, Sipoo, Finland	**	K13-5 (831109.1.1)

* Isolates obtained from spore prints kindly supplied by D. A. Reid.
** Finnish isolate obtained from a spore print kindly supplied by K. Korhonen; original code in parentheses.

Table 2.4. Collection sites and isolate codes for S. insignitum.

Date of collection	Substratum and site description	Supplier of spore print or basidioma	Codes of isolates obtained with supplier's code in parentheses.
8.x.80	Unknown	J. Boidin	B1-2 (LY9625)
Unknown	Beech (F. sylvatica), Forêt de Fontainbleau, France	P. Lanquetin	LQ1-15 (LY10348)

Table 2.5. Collection sites and isolate codes for S. rugosum.

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
14.iii.82	Fallen birch (Betula sp.) trunk, Clifford Farm, River Teign Valley, Devon	SX783896	RT12-6
17.iv.82	Alder (Alnus sp.) coppice stools, Highgate Common, S. Staffs	SO835904 *	H1-15, H2-15
13.vi.82	Hazel (C. avellana) coppice pole, Avon Gorge, Avon	ST563732	Q1w
29.xii.82	Hazel (C. avellana) coppice pole, nr. Shelley, Suffolk	TM021377 *	T1w
14.i.83	Hazel (C. avellana) coppice pole, River Avon valley, Avon	ST779643	U2-6
30.iv.83	Hazel (C. avellana) coppice pole, nr. Winsley, Wilts	ST799597	W1w
9.x.83	Attached cherry-laurel (P. laurocerasus) branch, Roundway, nr. Devizes, Wilts	SU008632 **	RO1w, RO1-6

Table 2.5. (Continued).

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
23.x.83	Attached rhododendron (Rhododendron ponticum) branch, Longleat Park, Wilts	ST826424	LG1-6
29.xii.83	Fallen birch (Betula sp.) trunk, Great Hill, nr. Belmont, Lancs	SD657203	GH1-10
29.xii.83	Standing beech (F. sylvatica) trunk, Great Hill, nr. White Coppice, Lancs	SD636188	GH2-6
11.iii.84	Attached crack willow (Salix fragilis) branch, Charterhouse on Mendip, Somerset	ST508562	CH1-6
15.iv.84	Attached oak (Quercus sp.) branch, Rivington Reservoirs, nr. Chorley, Lancs	SD624148	CR1-6
15.iv.84	Attached holly (Ilex aquifolium) branch, site as for preceding entry	SD620143	CR2-6

Isolates obtained from fresh material kindly supplied by *R.C. Dryden and ** A.D.M. Rayner.

Table 2.6. Collection sites and isolate codes for *P. velutina*.

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
9.ii.82	Detached horse chestnut (*Aesculus hippocastanum*) bark, Farleigh Wood, Somerset	ST792560	F10w
9.ii.82	Beech (*F. sylvatica*) litter, site as for preceding entry	ST791560	F11w, F13w, F13-20
9.ii.82	Charred detached branch, site as for preceding entry	ST792562	F14w
30.vi.82	Beech (*F. sylvatica*) litter, site as for preceding entry	ST792561	F25w, F25-20, F27w, F27-13
18.viii.82	Beech (*F. sylvatica*) litter, Friary Woods, Avon	ST785589	Y3w, Y4w, Y8w
4.v.83	Beech (*F. sylvatica*) litter, University of Bath, Claverton Down, Avon	ST773647	U5w, U5-20
16.x.83	Beech (*F. sylvatica*) litter, Inwoods, nr. Bath, Wilts	ST798634 *	IN1-8
19.x.83	Sycamore (*Acer pseudoplatanus*) litter, Golf Course Wood, Bath, Avon	ST767653	G1-8

* Isolates obtained from fresh material kindly supplied by A.D.M.. Rayner.

Table 2.7. Collection sites and isolate codes for P. laevis.

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
10.v.83	Detached beech (F. sylvatica) branch, Cross Plantation, Manor Farm, Stockton, Wilts	ST973380 *	M1w, M1-20
15.v.83	Beech (F. sylvatica) litter, Conkwell Wood, nr. Bath, Avon	ST787617 *	CW1w, CW1-15

*Isolates obtained from fresh material kindly supplied by A.D.M. Rayner.

Table 2.8. Collection sites and isolate codes for *C. puteana*

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
3.x.82	Dead gorse (*Ulex europaeus*), Long Ashton, Avon	ST536708	LA1w
7.x.82	Beech (*F. sylvatica*) log, Forest of Dean, Gloucs	SO611144	D16w
22.x.82	Beech (*F. sylvatica*) log, Savernake Forest, Wilts	SU217648	SV1w
2.xi.82	Elm (*Ulmus* sp.) log, University of Bath, Claverton Down, Avon	ST773646	U1w, U1-15
19.x.83	Hawthorn (*Crataegus* sp.) stump, Bath Golf Course, Claverton Down, Avon	ST769648	U8w, U8-20
29.xii.83	Hawthorn (*Crataegus* sp.) stump, nr. Heapey, Lancs	SD599206	RC1w, RC1-15
8.i.84	Wet coniferous floorboards, Montpelier, Bristol, Avon	ST594745	RR1w, RR1-15

Table 2.9. Collection sites and isolate codes for *S. hirsutum*.

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
6.x.78	Birch (*Betula* sp.) log, Pojo, Finland	*	K7-6 (781006.5.6)
?.ix.80	Birch (*Betula* sp.) stump, Vantaa, Finland	*	K8t (800900.1.1)
1.ix.81	Birch (*Betula* sp.) log, Vihti, Finland	*	K6t (810901.1.1)
1.xii.82	Birch (*Betula* sp.) logs, Espoo, Finland	*	K9-6 (821201.2.1), K10-6 (821201.2.2), K11-6 (821201.23)
5.xii.82	Fallen birch (*Betula* sp.) trunk, Taivassalo, Finland	*	K12-6
2.x.81	Detached ash (*Fraxinus excelsior*) branch, Farleigh Wood, Somerset	ST792560	F1-15

* Finnish isolates and spore prints kindly supplied by K. Korhonen; original codes in parentheses.

Table 2.10. Collection sites and isolate codes for *S. gausapatum*.

Date of collection	Substratum and site description	N.G. References	Codes of isolates obtained
2.x.81	Detached oak (*Quercus* sp.) branch, Farleigh Wood, Somerset	ST792561	F3-20
8.iii.82	Fallen tree trunk, site as preceding entry	ST795560	F21w, F21-20

STORAGE OF CULTURES AND EXSICCATA

Monosporous mycelia, field isolates of wood or basidiomatal origin and synthesized secondary mycelia were cultured on 2% MA slopes for 2-3 days at 20°C in bijou bottles. Heavy grade water-white liquid paraffin of S.G. 0.860-0.890 (Fisons) was autoclaved at 121°C for 45 min, cooled to 20°C and added to fill the bottles which were then stored at 4-6°C. Subculturing from each bottle on to a 2% MA plate before further storage on slopes under fresh liquid paraffin was effected at *ca*. 6-monthly intervals. Spore deposits and basidiomatal exsiccata were sealed in plastic bags and stored at 4-6°C.

ASSESSMENT OF CULTURAL CHARACTERISTICS AND EXPERIMENTAL PAIRINGS

Cultural assessments and pairings were performed with inocula cut as 4mm or 6mm diameter cylindrical plugs from the margin of actively growing colonies on 2% MA plates. Single centrally placed inocula were cultured for assessment of colony characteristics on 14cm diameter 2% MA plates using the terminology of Stalpers (1978) and colour nomenclature of Rayner (1970). Colour names separated by the word to, e.g. buff to sepia, indicate the limits of the range present, whereas those separated by a hyphen refer to intermediate shades. Radial growth rates were calculated from vernier caliper measurements of two perpendicular colony diameters per plate of three replicate 14cm diameter 2% MA plate cultures per isolate. The first of these was recorded once the colony had attained a linear radial extension rate and subsequent measurements were made at 2-day intervals until the plate margin had been reached.

In pairings between monosporous isolates, the inocula were placed centrally ca. 1cm apart on 2% MA plates. The gap was increased to 4cm in pairings involving field isolates or synthesized secondary mycelia. Interactions and subcultures were observed after 8-10 days and thereafter at 1-week intervals until visible changes had ceased or 3 months had elapsed. Initial sib pairings of each species were repeated once or twice to detect any variation in the range of reactions upon which criteria for secondary mycelial establishment were based.

CONFIRMATORY TESTS

A range of paired primary mycelia were subcultured in three different ways to confirm or refute the suspected presence or absence of secondary mycelium (Fig. 2.1) as follows.

A colonized agar strip (ca. 5 x 30mm) including a portion of confrontation zone was removed from the chosen interaction and inoculated across the centre of a fresh 2% MA plate (Fig. 2.1 site W).

Three plugs (4-6mm diameter) were removed from central and marginal positions (Fig. 2.1 sites X,Y,Z) and each was inoculated at the centre of a fresh 2% MA plate. Plug inocula (4-6mm diameter) of the isolates constituting the original pairing were added at the periphery of these dishes so that each contained three different inocula lying on a straight line.

Three more plugs were similarly removed from central and marginal positions within the interaction and equidistantly inoculated on the diameter of a fresh 2% MA plate as shown in Fig. 2.1.

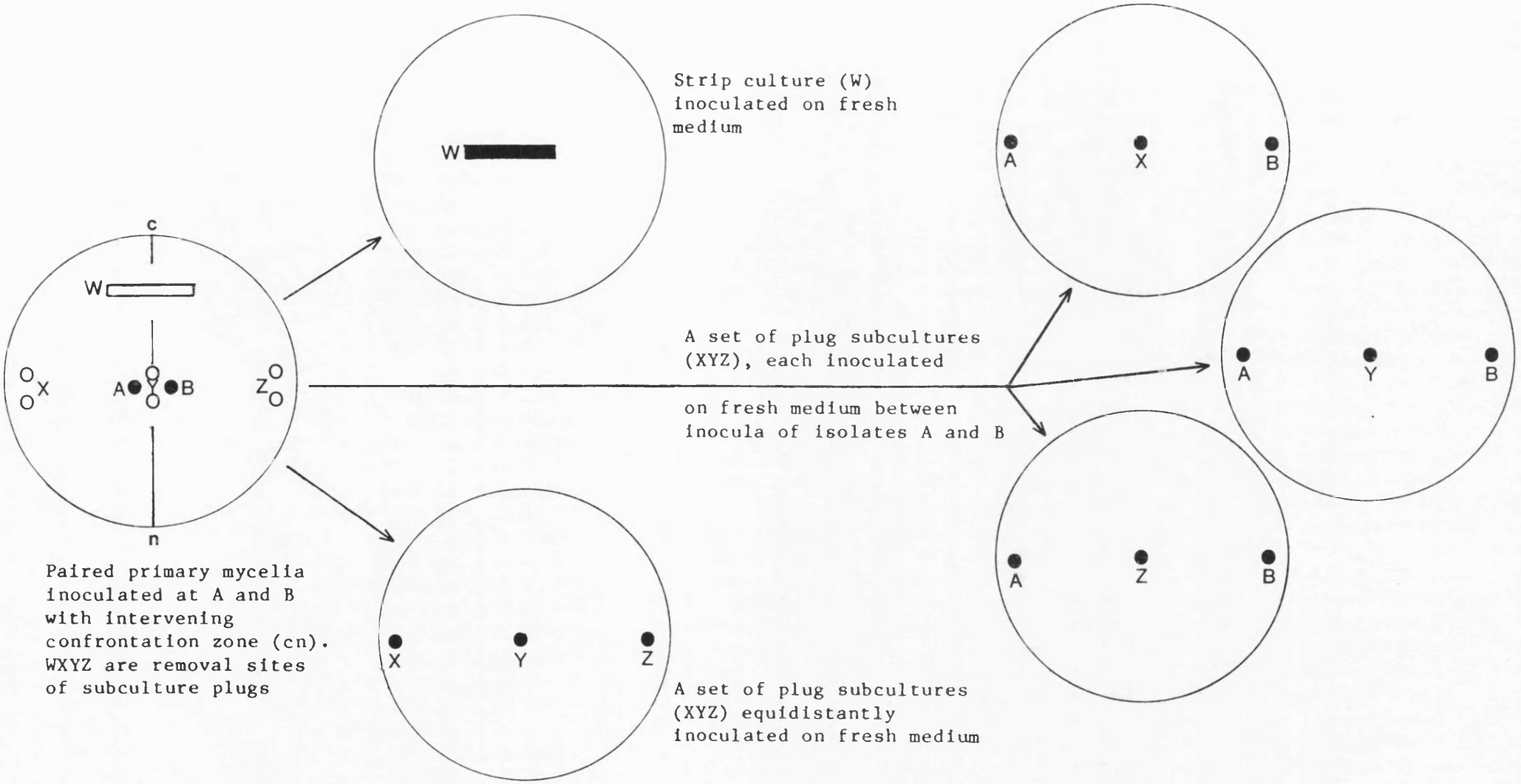


Fig. 2.1. Confirmatory testing of paired primary mycelia by strip and plug subculture and back-pairing of the latter against members of the original pairing. Closed circles and box represent inocula and open symbols represent removal sites from where such inocula were taken.

CHAPTER 3 POPULATION VARIATION AND ITS BASIS IN *S. sanguinolentum* AND *S. "rameale"*

INTRODUCTION

As a result of a previous study (Rayner & Turton, 1982) it was suggested that woodland populations of both *S. sanguinolentum* and *S. "rameale"* consist of interaction groups comprising closely or clonally related genotypes, such that members of a group intermingle in culture, but inter-group pairings exhibit somatic (heterogenic) incompatibility. However, the study of *S. "rameale"* was limited to a single basidioma and its progeny set and it was therefore necessary in the present investigation to make further isolations from British sites (Table 2.1) to check whether heterogenic incompatibility could be used to assess the population structure of this species.

By contrast, Rayner & Turton (1982) studied *S. sanguinolentum* more intensively using a sample of 14 basidiomata from a single larch (*Larix* sp.) stand. Hence it was necessary in the present investigation to extend the population survey of this species to provide a better understanding of geographical variations (Table 2.2). This included several collections from Finland since the cultural interactions of these had suggested that a similar pattern of population variation existed there as in Britain (Korhonen, pers. comm.; cited by Rayner & Turton, 1982).

MATERIALS AND METHODS

SIB PAIRINGS

The British collections of S. sanguinolentum (A1) and S. "rameale" (Y1), initially made in the present study, were each used to obtain 15 monosporous mycelia. For each progeny set, pairings were made in all combinations including control pairings of inocula from a single isolate. Following these initial studies, which confirmed that monosporous progeny from a basidioma were probably clonal, five or six sib primary mycelia were obtained thereafter from S. "rameale" collections and from the next 39 collections of S. sanguinolentum, all of which were subjected to the same pairing regime. The remaining collections of the latter species each provided three primary mycelia (Tables 2.1 & 2.2).

PAIRINGS INVOLVING FIELD ISOLATES

Wild basidiomata found to be touching but not confluent were removed with attached bark to expose luteous to orange (S. sanguinolentum) or pure yellow to luteous (S. "rameale") interaction zones in the wood beneath. Field isolates were obtained from both sides of these zones and paired to test whether a similar demarcation of interactive origin occurred in culture.

The first six field isolates to be obtained for each species, including those of Finnish origin, were paired in all intraspecific combinations which were compared with control

pairings of inocula from the same isolate. Field isolates were also paired with members of their progeny sets. When this involved three sibs only (S. sanguinolentum) the sib inocula were placed equidistantly around the periphery of a 2% MA plate with the field isolate inoculum located centrally. Control plates of sib trios, lacking a central inoculum, were also set up.

NON-SIB PAIRINGS AND ALLOCATION OF PRIMARY MYCELIA TO INTERACTION GROUPS

Initially a representative primary mycelium was chosen from each progeny set and paired in all non-sib combinations with representatives of successively isolated sets, using sibs in control pairings. Intermingling non-sibs could then be assigned to a common interaction group; thereafter a single or few primary mycelia per interaction group (tester isolates) were chosen for storage under oil.

In S. "rameale", tester isolates were inoculated equidistantly around the periphery of sets of two 2% MA plates (rapid test plates) to reduce the time and materials involved in testing each newly acquired collection. One of each set was a control plate and the other was centrally inoculated with a freshly isolated progeny set representative. Thus a primary mycelium could be simultaneously tested for membership of several interaction groups.

In S. sanguinolentum, a preliminary non-sib pairing regime was followed to establish the number of interaction groups at each site. Subsequently, group representatives were tested against a maximum of five tester isolates per rapid test plate.

When five tester isolates emerged as representing the most frequent interaction groups, they were used in rapid tests against freshly isolated primary mycelia before those of interaction groups with a smaller membership. The procedure for assigning S. sanguinolentum isolates to interaction groups is summarized in Fig. 3.1.

PAIRINGS INVOLVING PROGENY OF LABORATORY-PRODUCED BASIDIOMATA

In many cases, primary mycelia of S. sanguinolentum and S. "rameale" readily produced basidiomata in laboratory culture. For each species, three Petri dishes, each containing a fruiting isolate, were inverted and basidiospores collected on 9cm diameter cellophane discs, from which solitary germlings were obtained as described in Chapter 2. Six sibs were obtained per fruiting isolate and paired in all combinations, against their parental fruiting isolate and, where applicable, with their grandparental (i.e. field) isolates.

Basidiospores of each species were also collected from three cultures of paired isolates belonging to different interaction groups to test whether the interactional behaviour of the progeny was similar to that of an isolate present in the fruiting pairs. Six different sib inocula from each progeny set were placed singly and centrally on 2% MA plates and for each, stored culture inocula of both isolates present in the fruiting combinations were placed one on each side and ca. 3cm distant such that the three inocula per dish lay on a straight line (Fig. 3.2).

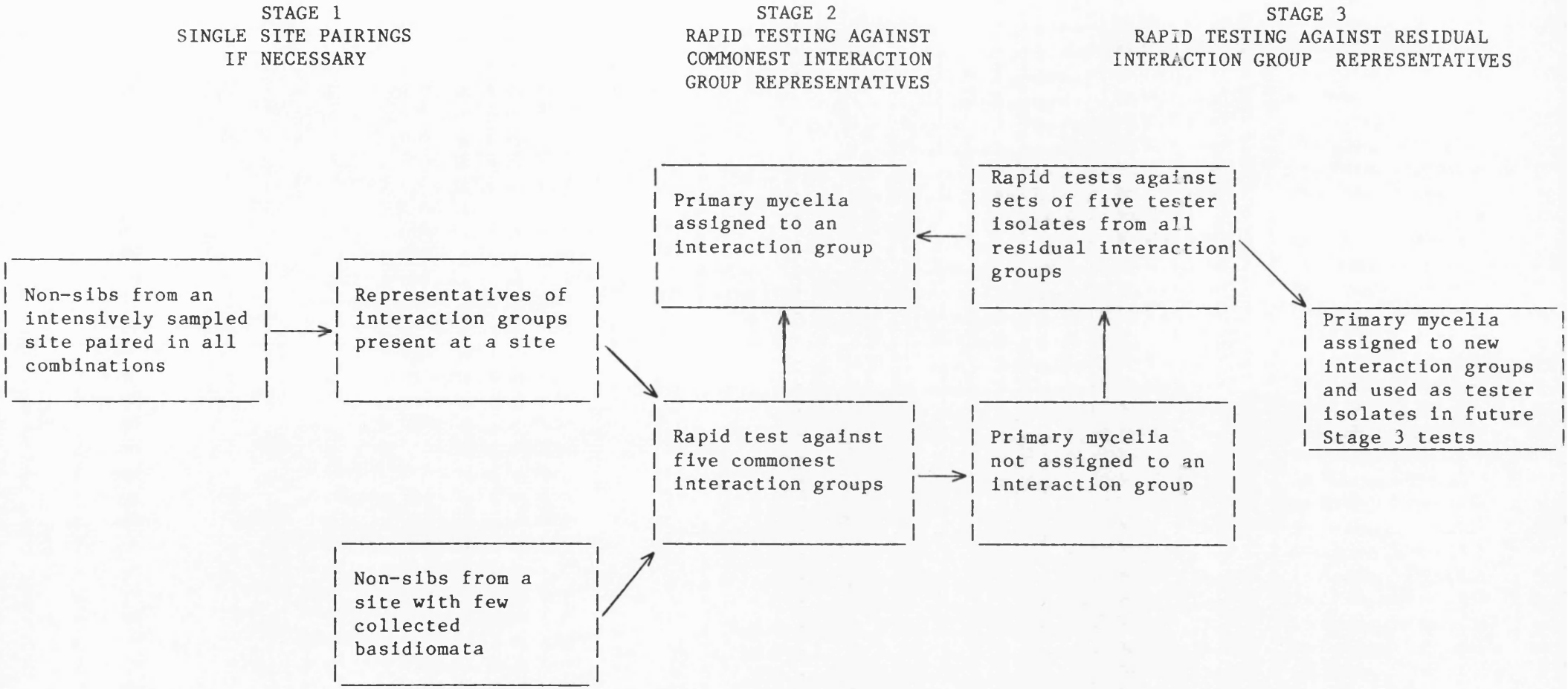


Fig 3.1. Triple stage procedure for allocating primary mycelia of S. sanguinolentum to interaction groups.

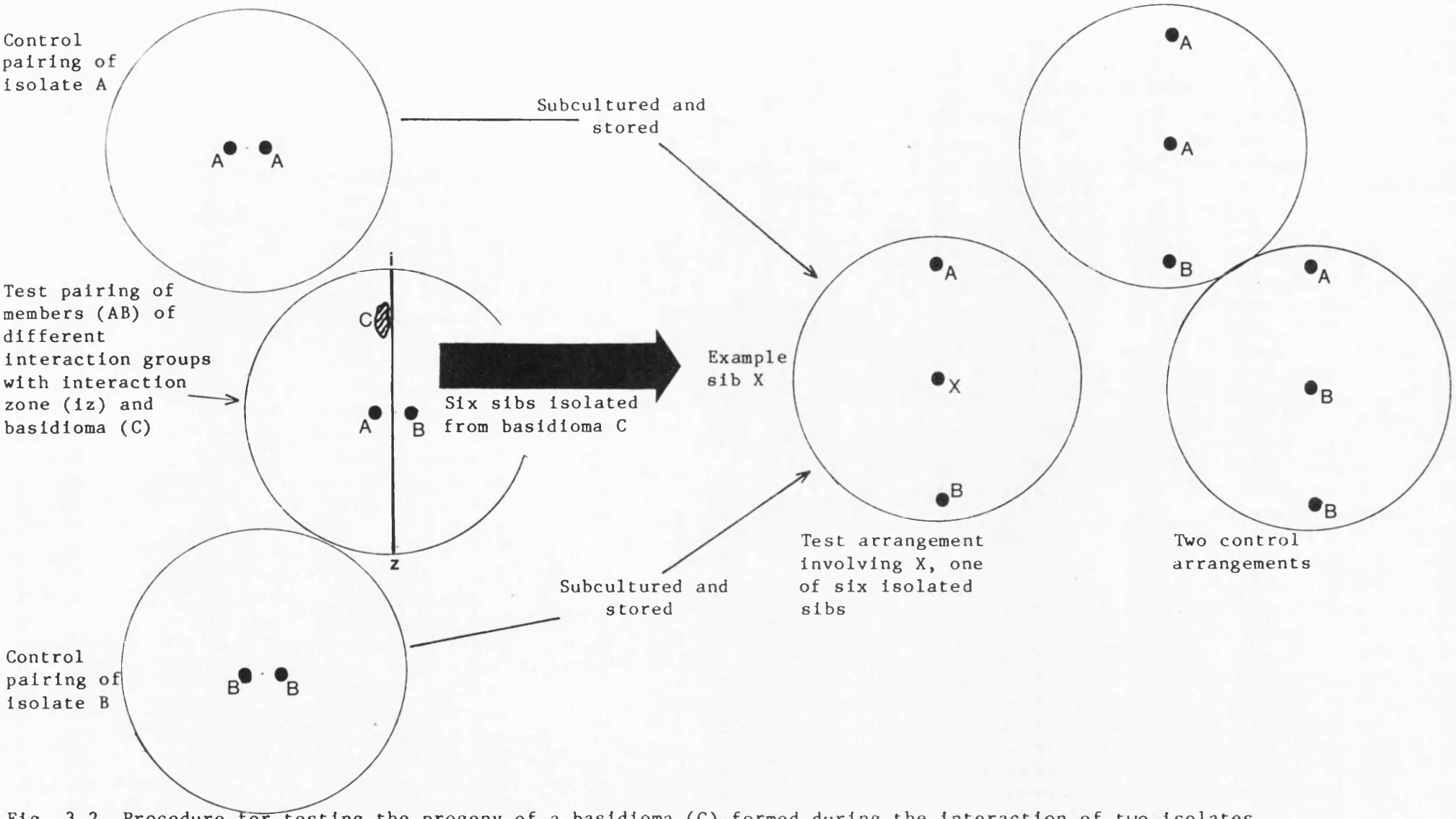


Fig. 3.2. Procedure for testing the progeny of a basidioma (C) formed during the interaction of two isolates (AB) from different interaction groups. X is one of six sibs tested against A and B to determine if either is parental. Closed circles represent inocula.

CONFIRMATORY TESTS

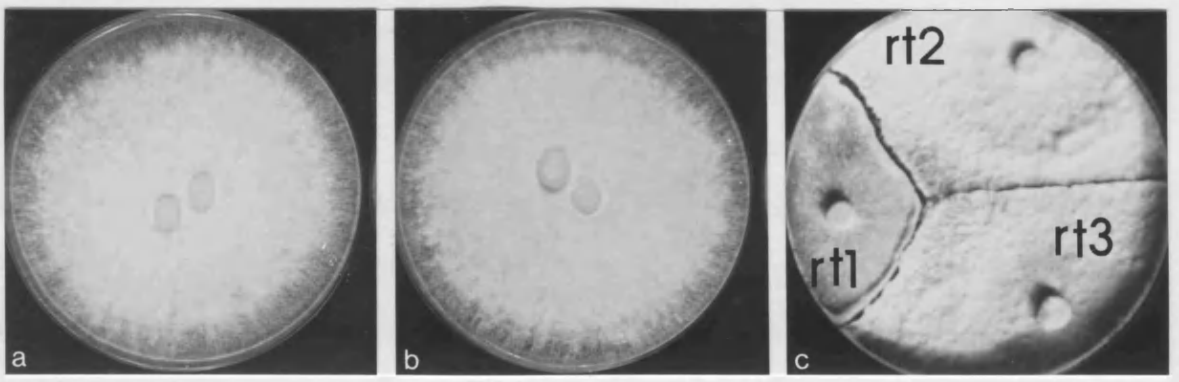
The procedure outlined in Chapter 2 was adopted for a selection of pairings of *S. sanguinolentum* including all those in which the constituent isolates remained slightly morphologically distinct, but failed to produce an interaction zone, and those inter-group pairings in which an apparent "partial replacement" (*sensu* Rayner & Turton, 1982) occurred.

RESULTS

CULTURAL CHARACTERISTICS OF *S. "rameale"*

With the exception of two collections (Y2, FA1) all isolates had morphologically similar white mycelial mats (Fig. 3.3a) which became centrally lacunose. An annulus of buff to sepia stromatic basidiomata usually developed around the edge of the Petri dish after 6-7 weeks incubation, of which the final 3-4 weeks were at room temperature (18-25°C) subjected to natural and fluorescent room lighting rhythms. Progeny of Y2 and FA1 showed wider variation in radial extension rates, mycelial density and degree of colony margin lobing such that slowly extending isolates had relatively dense submerged mycelia with strongly lobate margins. Microscopically these lobes consisted of interwoven, frequently branched, fine hyphae with few wider clamp-bearing elements. Basidiomata were only produced by relatively rapidly extending Y2 and FA1 progeny with frequently clamped wider hyphae and a lack of marginal lobing.

Fig. 3.3. (a-c) Mycelial interactions in S. "rameale" showing intermingling in (a) control pairing of different parts of a single primary mycelium (b) sib pairing and rejection (c) between members of three interaction groups. (d) Sketch map showing the distribution of detected interaction groups.


a
b
rt2
rt1
rt3
c


200 km
rt2
rt1
rt2
rt2
rt2
rt1,2,3,4,5
rt1
d

Occasionally, sites on a lobed colony margin would initiate "point growth" phenomena, i.e. a localized switch to a sparser mycelial density with an increased rate of extension (see Coggins et al., 1980). The resultant fan-shaped regions of mycelium with an increased frequency of whorled clamp connections would sometimes meet and intermingle, particularly when constrained by the edge of the dish. This contrasted with the relatively infrequent meeting of lobes which resulted in a further reduction of their extension rate, apparently associated with an intervening zone of antibiosis. Such delimitation (staling reaction) was also observed between paired, slow-growing and genetically identical secondary mycelia of P. betulinus and attributed to the release of self-inhibitory waste products (Adams, 1982).

EXPERIMENTAL PAIRINGS OF S. "rameale"

Sib pairings and pairings between parental isolates and their progeny

Field isolates intermingled with themselves in control pairings and with members of their progeny sets. The interactions of Y2 sibs and Y2w against its progeny were the most protracted, involving a preliminary hyaline intervening zone into which hyphae of paired isolates slowly extended before intermingling was evident.

Other sib pairings intermingled (Fig. 3.3b) and were indistinguishable from control pairings and from pairings of field isolates against their progeny, with the exception of FA1

isolates. The confrontation zones of the latter resembled those between Y2 sibs, however complete intermingling only took place between rapidly extending fans, originating by "point growth", and frequently-clamped non-lobate isolates. Otherwise a region of relatively sparse interdigitating hyphae persisted, which was sometimes associated with patches of pure yellow pigment. Consequently Y2 and FA1 sibs with the highest extension rates were chosen for non-sib pairing experiments.

Intermingling was also observed in pairings of laboratory-fruiting isolates against their progeny, sib pairings within such progeny sets and the interactions of progeny against grandparental field isolates.

Interactions of non-sibs, field isolates and resultant progeny

Intermingling non-sib isolates were assigned to interaction groups (Table 3.1) and the geographical distribution of five detected groups was plotted on a sketch map (Fig. 3.3d). Members of different interaction groups produced intervening zones of rejection (Fig. 3.3c) when inoculated on the same plate. The morphology of such zones ranged from a narrow $\leq$ 2mm superficial band of luteous to pure yellow pigmentation overlying lytic patches to a 4mm wide pure yellow lytic zone in which aerial mycelium was extremely sparse. The interactions of paired field isolates resembled those of non-sib combinations between their respective progeny sets. In one example (Y13wa x Y13wb) this revealed that members of two interaction groups had produced touching but non-confluent basidiomata on a single oak (Quercus sp.) branch.

Progeny of basidiomata developing in inter-group pairings always intermingled with one isolate of the pair and reproduced

Table 3.1. Membership of five interaction groups of S. "rameale" comprising isolates from twenty basidiomata collected from various angiospermous substrata.

Interaction group code	Isolate codes and substrata from which basidiomata were collected			
	Oak (Quercus)	Cherry-laurel (P. laurocerasus)	Blackthorn (P. spinosa)	Birch (Betula)
rt1	ST2,Y2,Y11,Y12,Y13a	PU1	TG1	AR1
rt2	AW1,CP5,L2,ST1,PF4, WT1,F29	SB1,FA1	---	---
rt3	Y1	---	---	---
rt4	Y13b	---	---	---
rt5	F28	---	---	---

the morphology of the inter-group interaction zone against the other isolate.

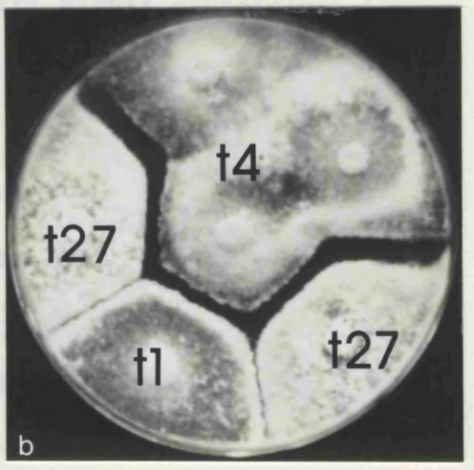
CULTURAL CHARACTERISTICS OF S. sanguinolentum

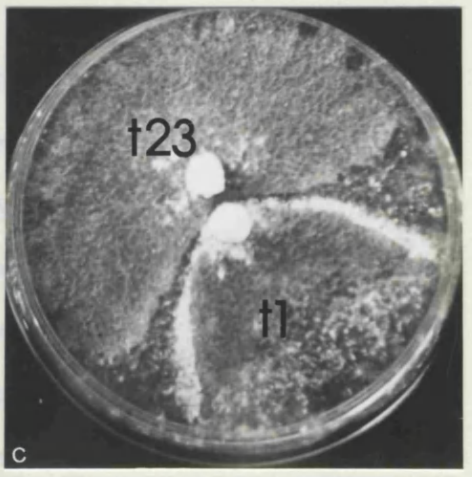
In contrast to S. "rameale", isolates of S. sanguinolentum exhibited a wide range of morphological variation (Fig. 3.4a) with radial extension rates in the range 3.3-5.1 mm day^{-1} such that rapidly extending isolates were hyaline or white, whereas slower ones were pigmented pale luteous to bay (c.f. similar observations by Rayner & Turton, 1982). Some isolates produced crustose zones (Fig. 3.4a) resembling pseudosclerotial plates (see Lopez-Real, 1975) within the colony which consisted of a mound of aerial mycelium and an underlying band of orange to scarlet pigmentation.

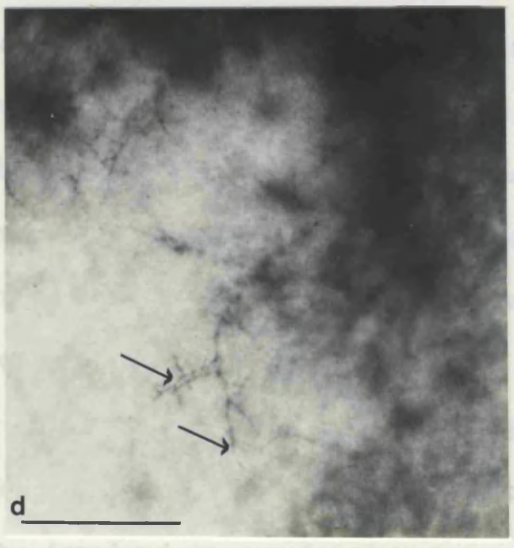
Field isolates and their respective progeny sets were usually lacking in variation although slight differences in pigmentation, radial extension rate and aerial mycelial density occurred within a minority. This variation remained after subculturing, but was not always isolate specific. The morphology of a single primary mycelium (A1-3) was exceptional in that it was grossly dissimilar to its 14 isolated sibs. It bore a strong resemblance to Y2 and FA1 progeny of S. "rameale" in that it had a relatively low radial extension rate, "flat" aspect, slightly lobed colony margin and apparently no clamp connections. However, unlike any S. "rameale" primary mycelium and any of its sibs, A1-3 was also strongly pigmented luteous to bay. The intensity of this pigmentation then declined in an inverse relationship with radial extension rate and aerial mycelial density with repeated subculturing.

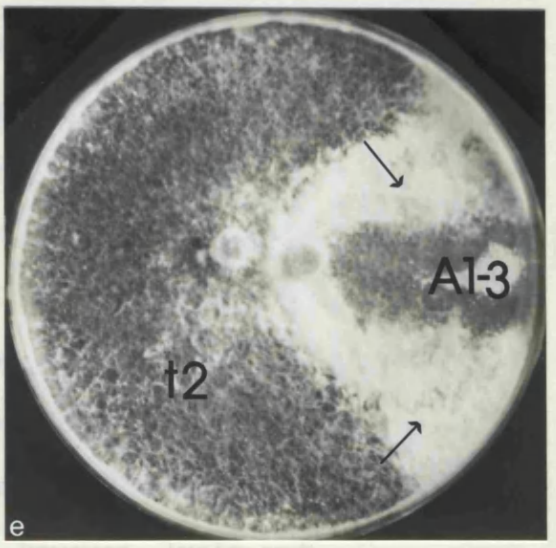
Fig. 3.4. (b-e) Mycelial interactions of S. sanguinolentum and (a) range of morphological variation; cz, crustose zone. (b) Rapid test plate showing intermingling of three non-sib-related primary mycelia in interaction group t4 and variation in width of inter-group rejection zones. (c) Bow-tie reaction between members of groups t23 and t1. (d) Rejection between t1 and t8 showing pigmented hyphae (arrowed) extending from the confrontation zone; bar marker represents 5mm. (e) Mycelial replacement (arrowed) of A1-3 by a member of t2.


a
c z


b
t4
t27
t1
t27


c
t23
t1


d


e
A1-3
t2

Laboratory-produced basidiomata were stromatic, vinaceous buff to brown vinaceous and bruised red-scarlet. Although occasionally developing at the edge of Petri dishes containing pigmented isolates, they were more frequently produced along the margins of interaction zones.

EXPERIMENTAL PAIRINGS OF S. sanguinolentum

Sib pairings and pairings between parental isolates and their progeny

Field isolates intermingled with themselves in control pairings and with members of their progeny sets. Likewise sibs intermingled with themselves in control pairings and also when paired against each other. Where there was a slight variation in morphology within progeny sets and/or between them and their respective field isolates, it was manifested in paired isolates in one of three ways: the differences remained even after subculturing; the morphology of the pairing approached uniformity which was retained after subculturing; the differences remained, but were reduced to almost zero after subculturing. The latter resulted in a near-uniform morphology which was either indistinguishable from that of one of the paired isolates or characteristic of the particular combination.

The isolate A1-3, although remaining morphologically distinct when paired with its sibs, nevertheless did not seem to be involved in a rejection response. However, lysis and pigmentation at the confrontation zone would be at least partially obscured by A1-3 mycelial morphology. This uncertainty

over its recognition capacity, together with its early date of collection, mitigated for the exceptional retention of two sibs from A1, i.e. A1-1 and A1-3, for non-sib pairings.

Sib pairings of laboratory-produced basidiomatal progeny intermingled and this response was also elicited between such progeny members and their parental isolates or grandparental field isolates.

Interactions of non-sibs, field isolates and resultant progeny

The majority of non-sib interactions resulted in an intermingling response or strongly pigmented rejection zone which facilitated the allocation of isolates to 31 interaction groups (Fig. 3.4b; Table 3.2) whose British distribution was plotted on a sketch map (Fig. 3.5). However, groups t5, t6 and t31 were exceptional in that t31 members intermingled with those of the other two. After 10 days incubation, the single member t6 group intermingled with t5, but after a further 3 weeks, narrow (ca. 1mm) pale apricot interaction zones had developed. On the basis of their interactions with all the tester isolates, three groups of intermingling members were recognized and their interactions are summarized in Fig. 3.6. The interactions of t23 against six members of t1 and three of t2 were also exceptional (Fig. 3.4c; 3.6) in that two types of response were observed within each set of combinations. These responses consisted of a confrontation zone that was either weakly pigmented pale apricot or hyaline and "blotchy". Microscopically, the latter comprised frequently-branched, intertwined wide (ca. 10μm) hyphae and this morphology also extended in an annulus around the paired cultures. Hence

Table 3.2. Membership of 31 interaction groups of *S. sanguinolentum* comprising isolates from 98 basidiomata collected from various gymnospermous substrata.

Interaction group code	Isolate codes and substrata from which basidiomata were collected			
	Larch (*Larix*)	Spruce (*Picea*)	Pine (*Pinus*)	Douglas fir (*Pseudotsuga*)
t1	SW1a,b,SW6,RT1	RH4,D6,D7,D8,D11a,HM4	A7,A10,S1,U7b,L1	---
t2	RH3,SK1,CP1	D3	---	---
t3	RT7,RT10	---	HM2	---
t4	A8,SW2,SW7,SW8b,SW13, SW14,SW15,SW16,SW20, RT4,RT6,RT9,SK3,WA1	D9	A5,A6,D5	---
t5	SW4,Y6,GC1	CP3,CP4	---	---
t6	SW19	---	---	---
t7	SW25	---	---	---
t8	SW10,SW17,SW21,SW22, SW24,RT3	D4,D11b	PF2	---
t9	A4,RH1,SW8a,SW9,SW12, RT5, SK2, SK4, Y7	D10	Y9,U7a,PF1	F30
t10	SW3	---	---	---

Table 3.2. (Continued).

Interaction group code	Isolate codes and substrata from which basidiomata were collected			
	Larch (*Larix*)	Spruce (*Picea*)	Pine (*Pinus*)	Unknown
t11	A1 (excluding A1-3)	---	R1,R2	---
t12	A1-3	---	---	---
t13	RH2	---	A3	---
t14	---	K1	---	---
t15	---	K2	---	---
t16	---	K3	---	---
t17	---	K4	---	---
t18	---	K5	---	---
t19	RT2	---	---	---
t20	RT8	---	---	---
t21	D1	---	---	---
t22	---	---	D2	---
t23	---	BF2	---	---

Table 3.2. (Continued).

Interaction group code	Isolate codes and substrata from which basidiomata were collected			
	Larch (Larix)	Spruce (Picea)	Pine(Pinus)	Unknown
t24	---	CP2	---	---
t25	---	---	HM1	---
t26	---	---	HM3	---
t27	---	---	A9,A11	U3
t28	---	---	---	U4
t29	---	---	PF3	---
t30	---	---	U6	---
t31	WA2	BF1	A2	---

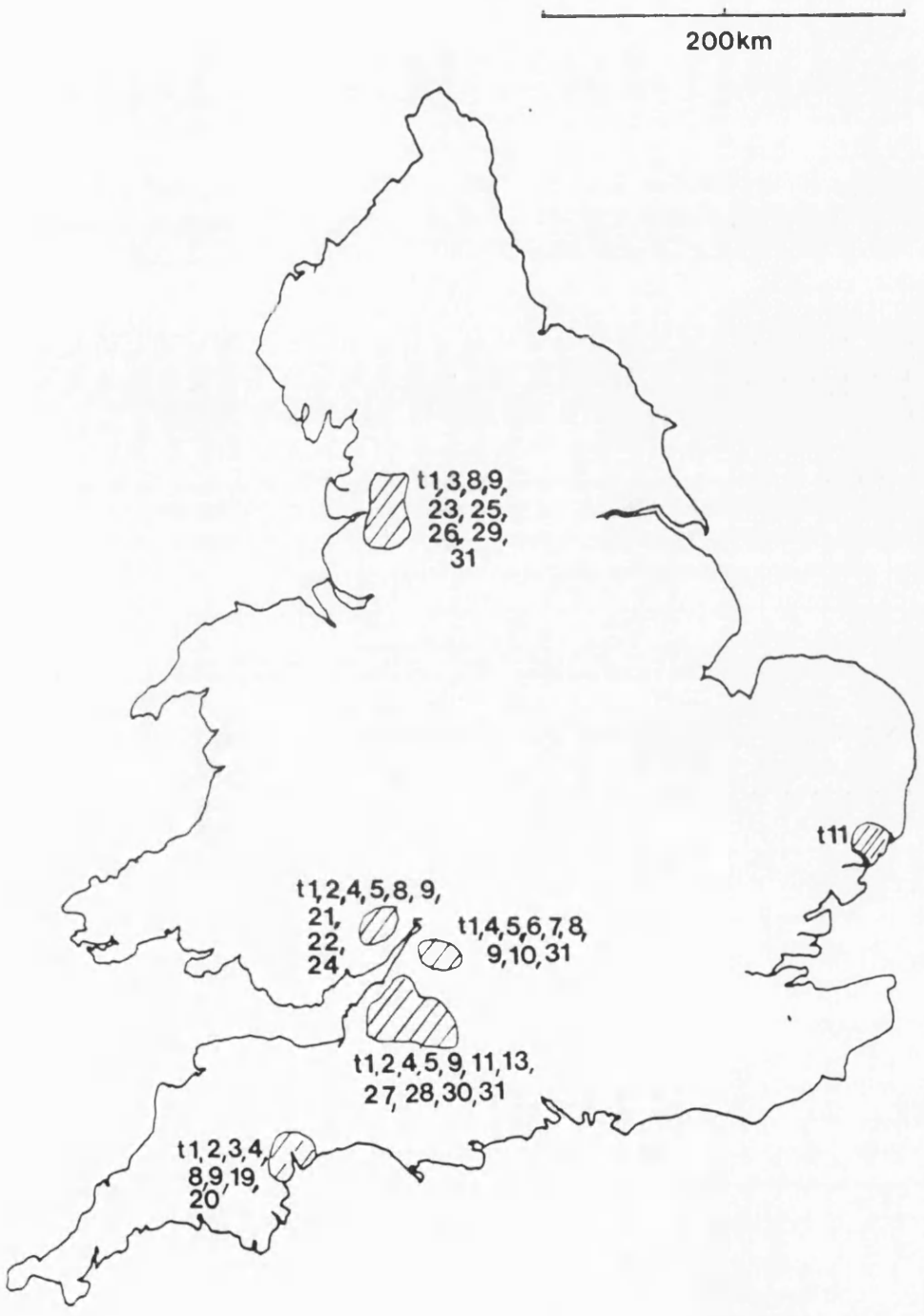


Fig. 3.5. Sketch map showing the British distribution of detected interaction groups in S. sanguinolentum.

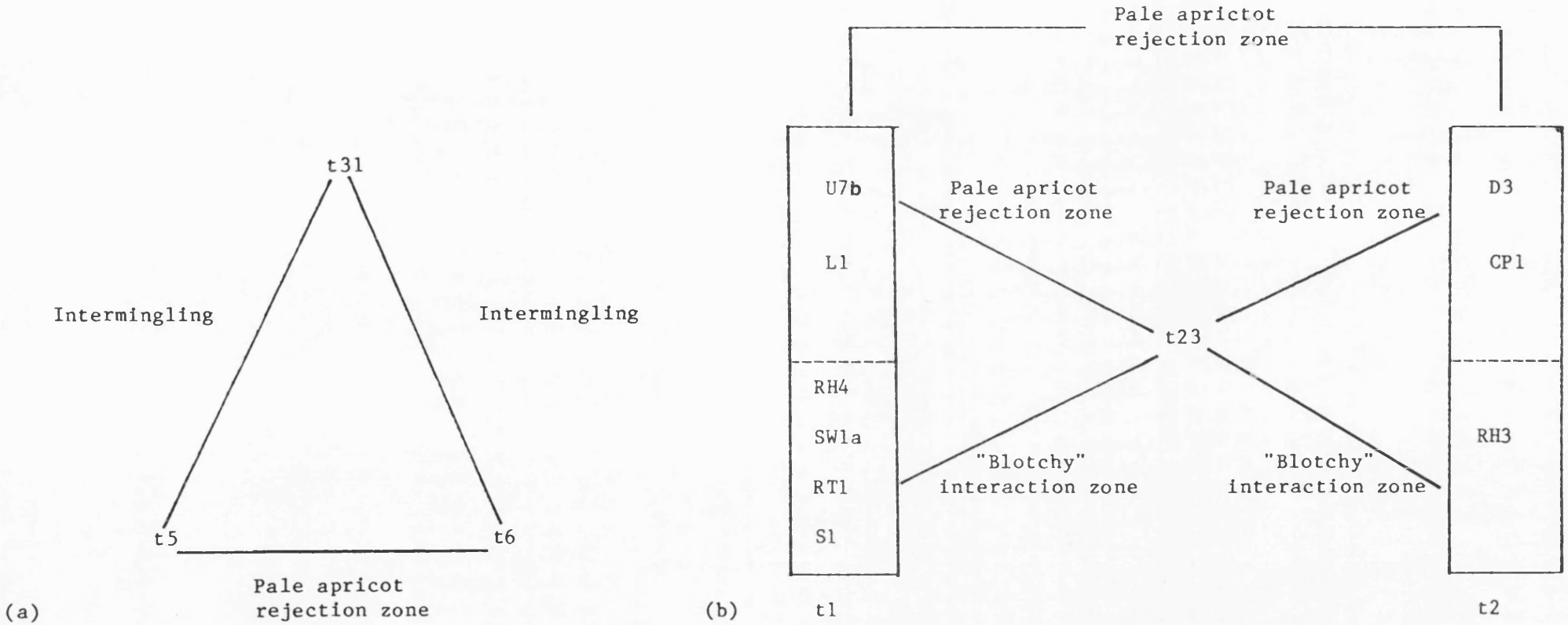


Fig. 3.6. Summarized interactions in S. sanguinolentum between (a) interaction groups t5, t6 and t31 (b) interaction group t23, six members of t1 and three of t2.

there are strong indications that t1 and t2 are not genetically homogeneous (clonal) and, using the criterion that interaction group members show similar responses in all non-sib combinations, both t1 and t2 could be split into smaller groups of intermingling members, had all the collections been retained.

The remaining inter-group reactions resulted in orange to blood coloured interaction zones, often widest ($\leq$ 2cm) at points distal to the inocula, in which the darkest pigmentation occurred in a band along the contact zone or flanked it on one or both sides when bulked ridges of aerial mycelium were present. Microscopically, the interaction zones contained an abundance of hyphal ghosts with granular, congealed pigmented contents. Several had a macroscopically fimbriate pigmented margin which was more easily observed from below in pairings of relatively unpigmented isolates. Microscopically, the fimbriae consisted of lengths of frequently-branched, intertwined pigmented hyphae, concolorous with the confrontation zone, and extending for up to 3.5cm from it (Fig. 3.4d).

Strip subcultures reproduced the morphology of the subcultured pairing, whereas plug subcultures removed from non-sib interactions intermingled with stock cultures of that member of the pairing whose inoculum was closer to the site of plug removal. Subcultures removed from the interaction zone itself developed in one of three ways: they reverted to the morphology of, and intermingled with one of the paired isolates; they reproduced the morphology of the subcultured pairing; or they retained the zone's pigmentation and generated a slowly extending lobed mycelium.

Subcultured examples of apparent "partial replacement" (sensu Rayner & Turton, 1982) always produced mycelia which intermingled with the isolate which had been originally inoculated on the subcultured side of the interaction zone. Isolates in which apparent "partial replacement" occurred usually produced pseudosclerotial plates when cultured alone which were morphologically similar to the bulked aerial mycelium which bordered the apparently replaced region. Whilst replacement was not indicated in such pairings, it did however occur in certain interactions of the anomalous sib A1-3 (Fig. 3.4e). Subculturing revealed that several isolates produced aerial mycelium which overarched the interaction zone and this was the only mycelial type to emerge from plugs removed from the A1-3 side of the pairing.

The interactions of paired field isolates corresponded with those of their respective progeny in non-sib pairings. Field isolates from touching but non-confluent basidiomata were assigned to different interaction groups, e.g. U7a,b and D11a,b, but those from basidiomata in close proximity on a single branch were either similarly assigned, e.g. SW8a,b, or placed in a single group, e.g. SW1a,b. It seems likely that the latter represented repeated sampling of a single mycelium.

Progeny of basidiomata located on the edge of the interaction zone in inter-group pairings intermingled with one isolate of the original pair and reproduced the original interaction zone against the other member of that pair.

DISCUSSION

RELATION TO PREVIOUS WORK AND ECOLOGICAL CONSEQUENCES

The work of Rayner & Turton (1982), which revealed interaction groups comprising intermingling field isolates and non-outcrossing primary mycelia of *S. sanguinolentum*, has been confirmed and extended to include *S.* "*rameale*". Since the assignment of isolates to interaction groups was based on a macroscopic intermingling response, it cannot be assumed that such an assay can distinguish entire genotypes (see pp. 34-35). Consequently Rayner & Turton (1982), in view of their growth rate data, indicated that a clonal or close relationship existed between group members. In support of the latter, slight morphological variation was detected within interaction groups in the present study. However, of more significance were the interactions between t5, t6 and t31 and between t1, t2 and t23 which imply that the intermingling response is determined by genetic similarity of heterogenic incompatibility factors rather than their identity. This contrasts with similar population studies of the reef-building coral, *Acropora cervicornis* (Neigel & Avise, 1983b) and seven tropical marine sponges (Neigel & Avise, 1985). In these studies, responses to vegetative grafting always showed transitive relationships, i.e. if A accepts B and B accepts C then, by transitivity, A should accept C.

In the absence of full DNA sequence data, genetic variation that does not contribute to the detectable manifestation of heterogenic incompatibility is probably best analysed by a combination of biometrical methods (e.g. Simchen &

Jinks, 1964; Brasier, 1970; Betterley & Collins, 1983; Lyman & Ellstrand, 1984) and isozyme comparisons (e.g. Spieth, 1975; Baptist & Kurtzman, 1976; Betterley & Collins, 1983; Lyman & Ellstrand, 1984; Old, Moran & Bell, 1984).

Two basic features of the present study were in accord with similar surveys of E. nidulans h-c groups (Croft & Jinks, 1977) and the corresponding O. ulmi v-c groups (Brasier, 1984). These studies demonstrated that member isolates of large h-c or v-c groups ("super-groups") inhabited widely separated sites and that large collections from a single site comprised members of many different groups.

Population structures of S. sanguinolentum and S. "rameale" also resembled those of American dandelions (Taraxacum) in which, despite the production of abundant pollen, sexual reproduction has not been reported. Solbrig & Simpson (1974) reported that three sites were inhabited by "multiclonal" T. officinale populations and in a wider survey, Lyman & Ellstrand (1984) found that 66% of dandelion "clones" were restricted to single collection sites, but a few "clones" had a very widespread distribution. The corresponding values for interaction groups of British S. sanguinolentum (excluding t12) and the much smaller survey of S. "rameale" are both approximately 60%, although the area of a site was rather arbitrarily chosen.

In Stereum, the majority of inter-group confrontations could be interpreted in terms of override failure, as originally proposed by Rayner & Turton (1982), involving direct rejection (heterogenic incompatibility) between non-sibs of sufficient genetic dissimilarity. An ecological corollary to this is that woodlands in which these species are well represented would be

expected to contain numerous spatially discrete mycelia ultimately derived from a common basidiospore source which intermingle in culture (see Rayner & Turton, 1982). Should such mycelia occur as neighbours in spatially continuous substrata, they may act synergistically, thereby losing individuality, but gaining co-operative resource capture capacity (Rayner et al., 1984) which would be particularly advantageous within an overall ruderal strategy (Cooke & Rayner, 1984). Although there is little evidence for such an ecological strategy in S. sanguinolentum and S. "rameale", it is nevertheless indicated by observations of their relative ease of fruiting in culture. Moreover, wild basidiomata are usually found on substrata which, by inference, have been available for saprotrophic colonization for a relatively short time, i.e. parts of trees that have been recently detached or wounded.

Likewise, Betterley & Collins (1984) interpreted co-operation among mass-spore-derived cultures of apomictic (asexual) isolates, compared with competition among those of heterothallic isolates, as conferring a selective advantage on the former in the production of myxomycete plasmodia. Indeed, apomixis is apparently the predominant breeding strategy in phaneroplasmodial myxomycetes in general. Furthermore, synergism of S. sanguinolentum mycelia may be naturally facilitated by an insect association, particularly with siricid wood wasps (e.g. see Talbot, 1964) which could repeatedly inoculate substrata with a high proportion of sib-related basidiospores and/or mycelial fragments.

Slowly extending, clampless lobed isolates were previously observed among primary mycelia of S. gausapatum (Boddy

& Rayner, 1982), some of which also exhibited "point growth" phenomena after storage. A degree of germling mycelial autoinhibition may be ecologically advantageous in module-mediated latent invasion during primary resource capture. This strategy involves the dispersal of modules, i.e. asexual spores or mycelial fragments, within at least partially functional wood elements. Subsequently they germinate into, or regenerate, intermingling mycelia effecting a much faster resource capture than would be possible by mycelial extension alone (see Boddy & Rayner, 1982; Rayner et al., 1984; Cooke & Rayner, 1984). It is also noteworthy that all germling mycelia in all members of Stereum considered herein initially lack wide clamp-bearing hyphae and generally have a relatively wide angle of branching (ca. 90°). Adopting Gregory's (1984) concept of mycelial modes, the clampless primary mycelial stage can be interpreted as a germling mode which for a minority of genotypes may extend throughout the primary phase.

EVIDENCE FOR ACCESS MIGRATION

The strongest indication that some non-sib interactions were involving nuclear and/or cytoplasmic exchange leading to heterokaryon and/or heteroplasmon initiation respectively, was provided by the "blotchy" regions within pairings of t23 against certain members of t1 and t2 (Fig. 3.4c). Such a distinctive hyphal morphology and its peripheral distribution within the Petri dishes may be evidence for unstabilized access migration (see pp. 49-52).

If override and access migration can occur in S. sanguinolentum (no comparable phenomena were detected in S. "rameale") then this implies the presence of at least a partially functional homogenic I.S.. Morphologically, t1, t2 and t23 members had similar radial extension rates and lack of pigmentation. It would be useful to investigate the levels of variation present within and between these groups to test the hypothesis that override occurs in S. sanguinolentum, but is restricted by the heterogenic I.S. to confrontations between genetically similar isolates. If this was so, then it may represent another example of an evolutionary trend to the imperfect (asexual) state seen in many Aspergillus spp. (Butcher, 1968).

The width of, and concolorous hyphal extensions from, certain pigmented interaction zones (Fig. 3.4d) may also be manifestations of unstabilized access migration. By effectively delaying rejection until numerous compartments have become heteroplasmons or, in the wake of septal erosion, heterokaryons; it could then be expressed over a wide area. This suggests that between conspecific primary mycelia, relatively narrow rejection zones indicate rapid compartmentalization of heterogenic incompatibility. In turn, this suggests that the degree of genetic dissimilarity between participants is outside the range that can be overridden by dissimilar mating-type factors.

This permits a re-interpretation of Brasier's (1984) data relating the degree of heterogenic (vegetative) incompatibility to the width of rejection zones in O. ulmi. Here it was argued that the widest zones occurred in "fully vegetatively incompatible" interactions and repeated back-crossing experiments

supported the view that decreasing genetic dissimilarity between members of a pairing resulted in narrower rejection zones. However, in terms of a fluctuating heterogenic and homogenic incompatibility concept (Fig. 1.1), wide zones might occur between isolates which are sufficiently genetically dissimilar to activate a mating-type independent override mechanism and not be the result of full heterogenic incompatibility *per se*.

In *S. sanguinolentum*, pigmented hyphae extending from the confrontation zone were prone to coiling, distortion and branch proliferation. This indicates that not only was the wall synthesis/lysis balance perturbed, but an extracellular recognition system might also be operative. The resultant intertwined hyphal morphology closely resembles that of certain mycoparasitic encounters, e.g. *Pseudotrametes gibbosa* on *Bjerkandera adusta* (Rayner & Todd, 1979) and *L. betulina* on *Coriolus* spp. (Rayner, Boddy & Dowson, 1986). Of greater relevance are Parag's (1970; cited by Nguyen & Niederpruem, 1984) and Nguyen & Niederpruem's (1984) observations of such hyphal morphology developing during intraspecific encounters within *S. commune*. The latter authors recorded entwining interactions during di-mon matings and noted that most hyphae in the zone of entrapment (at least two or three compartments) became senescent in the complete absence of anastomosis. In view of the common non-self acceptance nature of all these interactions, there is clearly scope for more detailed investigation of a possible link between post-access migration self/non-self recognition phenomena and non-self parasitic behaviour.

POSSIBLE ORIGIN OF *S. sanguinolentum* AND *S. "rameale"* POPULATION VARIATION

A widespread distribution of large interaction groups and the often relatively intense interactions between different groups at a single site suggest that the British population structure can be largely explained by woodland colonization occurring after the cessation of outcrossing. Whilst it is acknowledged that if such a barrier was imposed between a parental mycelium and meiotically-derived progeny, the resulting rejection would be relatively weak, nonetheless it seems *a priori* more likely that recent detection of such weak responses indicates post-outcrossing mutational divergence. Superimposition of such divergence is also suggested particularly in *S. sanguinolentum* by the existence of small site-specific groups and intransitive intermingling relationships. For example, before mutation t6, which only contains one isolate, may have intermingled with t5 and t31 which would then have constituted a single interaction group which is now in the process of splitting.

If *S. sanguinolentum* and *S. "rameale"* were once outcrossing species or have such a breeding strategy elsewhere, then the transition to a non-outcrossing strategy could perhaps be achieved by homokaryotic fruiting of primary mycelia exhibiting some characteristics of secondary mycelia. These characteristics have usually been attributed to the presence of unlike mating-type factors, in particular the propensity for donation of nuclei but not their receipt. Thus recombination would be precluded in such hypothetically functional, but not true, secondary mycelia and clones of offspring would result.

This resembles a model of the origin of a similar population structure in Daphnia pulex (Hebert & Crease, 1983) in which female meiosis fails to occur as a result of the action of a suppressor mutation which is transmitted through the gamodeme by male gametes. In the Stereum model, an equivalent role to that of male Daphnia gametes is also envisaged for the functional heterokaryons if the factor causing their simulated change of mode is transmissible. Furthermore, if it conferred selective advantages, e.g. rapid radial extension, early commitment to fruiting and progeny synergism, then the proportion of the species within the gamodeme may decline over evolutionary time and ultimately be replaced by non-outcrossing forms. It is generally believed however, that such forms, deprived of recombination, would gradually accumulate deleterious mutations and be less able to adapt to changing environmental conditions which, in the case of a parasite would mean cessation of co-evolution with its host.

It is of great interest that just such a cytoplasmically transmissible mode-switching factor has been reported in a primary mycelium of S. hirsutum following storage at 4-6°C under liquid paraffin (Coates & Rayner, 1985b). Furthermore, transmission of this factor to a sib-related mating-type incompatible isolate resulted in a bow-tie shaped interaction, indicating access migration, and rapid fruiting. Progeny of the isolate thus transformed were of two types, one of which was relatively slowly-extending, dense and had an abnormally branched morphology. This type then spontaneously reverted to a rapidly-extending effuse morphology and thus closely resembled the aberrant isolates of S. sanguinolentum and S. "rameale".

It seems imperative therefore to investigate whether a similar phenomenon could occur in a perennating homokaryon of an outcrossing form that is deprived of potential conjugants within a natural substratum - as may occur at the extremities of its geographical range. If a stress relationship could be demonstrated, then the rate of disappearance of an ancestral gamodeme may have increased with time as a result of increases in both transmission and spontaneous activation rate of a mode-switching factor.

CHAPTER 4 MYCELIAL INTERACTIONS WITHIN AND BETWEEN *S. subtomentosum* AND *S. insignitum*

INTRODUCTION

The two species *S. subtomentosum* and *S. insignitum* will be considered together in this chapter because it was apparent from the few basidiomata examined that they could not be easily distinguished in the field. This similarity was noted by Pouzar (1964) in his description of *S. subtomentosum* as a new species and, more recently, by Demoulin (1985).

Microscopically, *S. subtomentosum* lacks pseudoacanthophyses (pseudoacanthohyphidia), i.e. lanceolate paraphyses with minute aculeolate projections, in the hymenium which distinguishes it from *S. insignitum*, e.g. Jahn's (1971) key to European members of the genus and Jülich & Stalpers' (1980) key to *Stereum* spp. of the temperate Northern Hemisphere. This difference has also been incorporated into criteria delimiting three subgenera (Boidin *et al.*, 1979) which have recently been used in a key to North American species (Chamuris, 1985b). Thus *S. subtomentosum*, *S. hirsutum* and *S. gausapatum* have been placed in subgenus *Stereum* whereas *S. insignitum*, *S. sanguinolentum* and *S. rugosum* belong to subgenus *Aculeatostereum*. Since this distinction has only been made relatively recently, care must be exercised when surveying the older literature, for example Boidin & Lanquetin (1984c) state that *S. insignitum* (*sensu* Boidin, 1950, 1958) referred to *S. subtomentosum* as it is currently understood. In view of this confusion, the current investigation sought to

compare cultural characteristics and mycelial interactions with existing taxonomic criteria.

S. subtomentosum - MATERIALS AND METHODS

Fifteen sibs and a tissue isolate were obtained from F5 and paired in all combinations. This regime was also followed for the E1 progeny set of 15 and each of the KW1 and K13 sets of five.

As a result of these interactions, a single isolate was selected from each set and paired against F5t and in all possible non-sib combinations. Six primary mycelia were obtained from each of three basidiomata situated near the confrontation zone of non-sib-related pairings. They were paired in all possible sib combinations, against their putative parental isolates and, when this included an isolate of F5 origin, against their grandparental field isolate F5t.

S. insignitum - MATERIALS AND METHODS

A French basidioma (LQ1) and spore print (B1) provided 15 and 2 sibs respectively and pairings were made in all sib and non-sib combinations. Two sibs of B1 produced basidiomata when cultured alone and each was used to obtain a further progeny set of six. These were paired in all sib combinations and back-paired against their parental isolates. Six interspecific pairings were also made between primary mycelia of S. subtomentosum and S. insignitum.

RESULTS

CULTURAL CHARACTERISTICS OF *S. subtomentosum*

The isolates were initially white with a farinaceous to granular aerial mycelium surrounding a felty central mat (Fig. 4.1a). Areas of pale luteous to luteous pigmentation developed in certain progeny sets, but were particularly intense and frequent within that of E1. Sibs were almost identical and the tissue isolate F5t was morphologically indistinguishable from its progeny. Globose, stromatic white basidiomata were formed abundantly in all cultures and showed the pure yellow bruising response of wounded wild hymenial margins. Microscopically, the mycelia were similar and bore whorled clamp connections on their wider hyphae.

EXPERIMENTAL PAIRINGS OF *S. subtomentosum*

Paired sibs from wild and laboratory-produced basidiomata intermingled in culture (Fig. 4.1b) and were indistinguishable both from control pairings and those of primary mycelia against their parental tissue isolate. Following the procedure adopted for *S. "rameale"* and *S. sanguinolentum*, each collection was assigned to a single-member interaction group.

Inter-group pairings produced narrow (1-2mm) luteous interaction zones which lacked aerial mycelium (Fig. 4.1c) and this result was also obtained when F5t was substituted for any of its progeny in non-sib combinations. Progeny of basidiomata derived from non-sib pairings intermingled with one member of the

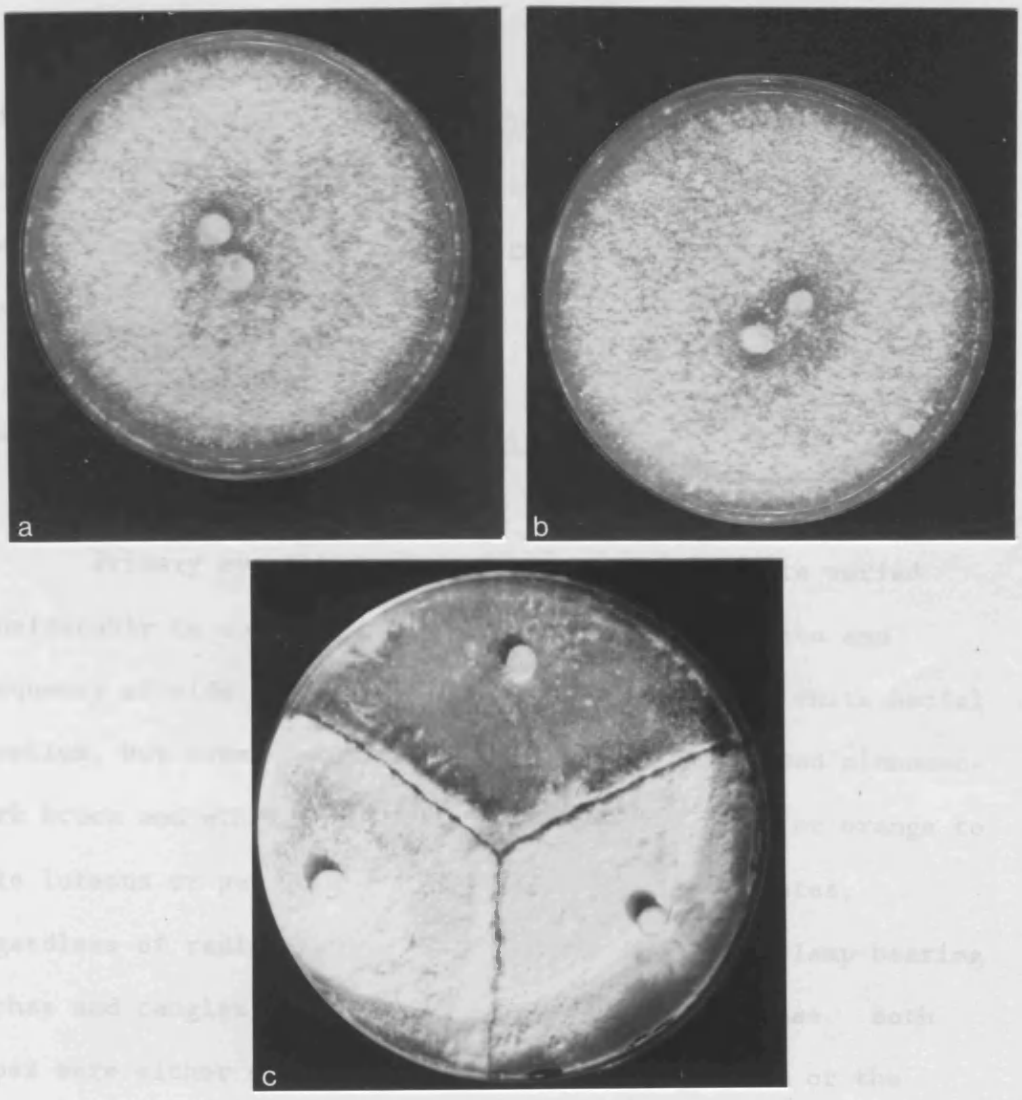


Fig. 4.1. Mycelial interactions in S. subtomentosum showing intermingling (a) between different parts of a single primary mycelium (b) between sibs and rejection (c) between members of three interaction groups.

original pairing and produced similar non-sib interaction zones against the other member. In addition, laboratory-produced progeny of F5 primary mycelia intermingled with their grandparental F5t isolate.

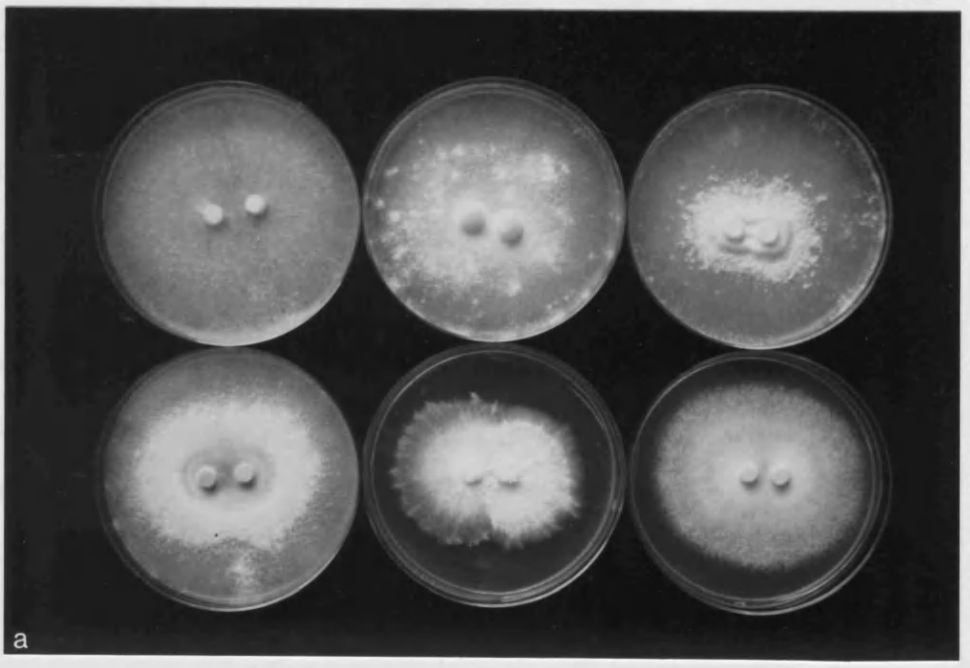
CULTURAL CHARACTERISTICS OF *S. insignitum*

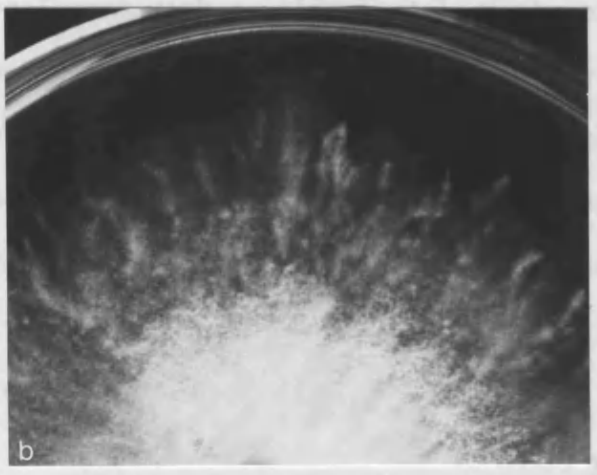
Primary mycelia derived from wild basidiomata varied considerably in colour, texture, radial extension rate and frequency of wide clamp-bearing hyphae. Many had a white aerial mycelium, but some were partially or totally pigmented cinnamon-dark brick and others had annuli which were saffron or orange to pale luteous or pure yellow (Fig. 4.2a). Most isolates, regardless of radial extension rate, had both wide clamp-bearing hyphae and tangles of profusely branched narrow hyphae. Both types were either evenly distributed in the mycelium or the former was restricted to regions with a relatively high rate of extension, apparently originating by "point growth".

Clamp connection frequency generally increased with subculturing, however four isolates remained clampless, of which two had the lowest radial extension rates (LQ1-1, LQ1-2). The latter had a very appressed margin containing linear aggregations of densely branched, submerged narrow hyphae (Fig. 4.2b). Macroscopically, these resembled mycelial cords and were interspersed with superficial regions of "point growth" which later obscured the underlying structures.

Fruiting was restricted to LQ1-9 and the primary mycelia of B1 which produced arcs of globose basidiomata. These were initially white, but became pure yellow then scarlet on bruising.

Fig. 4.2. Sibs of *S. insignitum* showing (a,b) range of morphological variation and (c,d) mycelial interactions. (c) Mating-type incompatible pairing showing rejection. (d) Control pairings of two primary mycelia (left and right dishes) and mating-type compatible pairing (centre dish) showing initial secondary mycelial establishment localized in a bow-tie shaped region (margins arrowed).


a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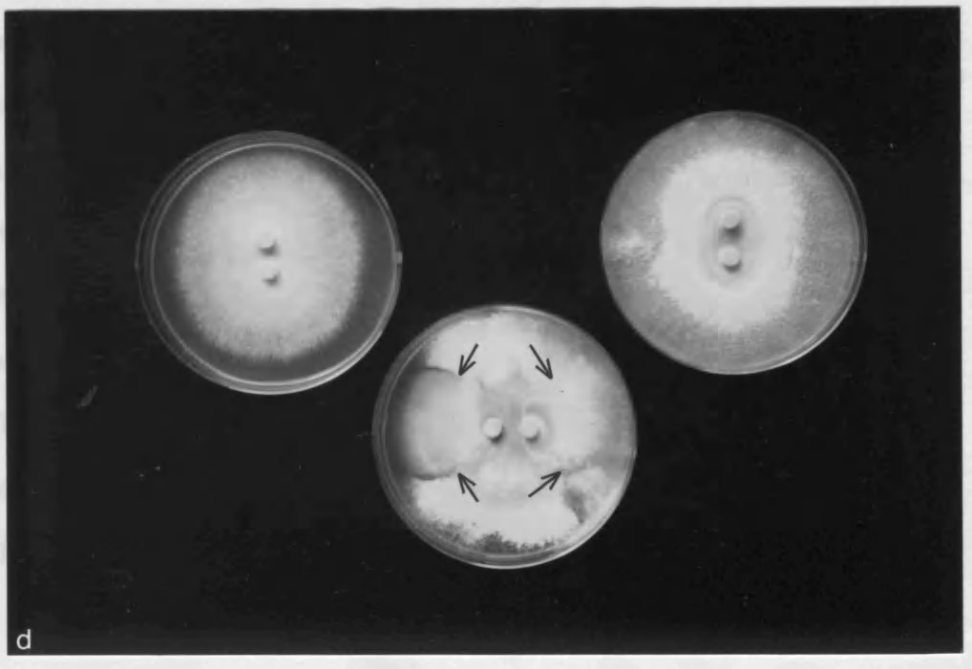

d

The resulting progeny were indistinguishable both from their sibs and their parental isolates.

EXPERIMENTAL PAIRINGS OF S. insignitum

Progeny of fruiting primary mycelia intermingled in both sib and sib/parent combinations. However, morphologically distinct, aerially dense, clamped mycelium developed between paired non-sibs and certain LQ1 sibs. Its manifestation in confirmatory tests was used as the criterion for secondary mycelial establishment and the resultant interaction pattern was consistent with a unifactorial diaphoromictic system of homogenic incompatibility (Table 4.1). Secondary mycelia were stable on subculture and pigmented pale luteous to ochreous or orange in their central regions with white marginal zones. One exceptional primary mycelium (LQ1-13) contributed to secondary mycelium development in all sib and non-sib pairings and so could not be assigned to either sib mating-type.

A similar pattern of secondary mycelial establishment occurred in all mating-type compatible pairings and was preceded by a bilaterally or unilaterally expanding mottled zone, whose features were consistent with access migration (see pp. 49-50; Fig. 4.2d). This originated within the confrontation zone and contained proliferations of finely branched hyphae associated with a lack of aerial mycelium. Such appressed zones were usually widest at the edge of the Petri dish and tufts of secondary mycelium arose therein, becoming confluent as establishment progressed. However a 1-5mm wide interaction zone often persisted at the interface of the secondary and resident

Table 4.1. Sib interactions of S. insignitum LQ1.

Isolate codes															
13	4	12	9	11	6	8	3	5	1	10	14	2	7	15	
I	C	C	C	C	C	C	C	C	C	C	C	C	C	C	13
	I	D	D	D	D	D	D	D	C	C	C	C	C	C	4
		I	D	D	D	B	D	D	C	C	C	C	C	C	12
			I	D	D	D	B	D	C	C	C	C	C	C	9
				I	D	D	D	D	C	C	C	C	C	C	11
					I	D	D	D	C	C	C	C	C	C	6
						I	D	C	C	C	C	C	C	C	8
							I	D	C	C	C	C	C	C	3
								I	C	C	C	C	C	C	5
									I	D	D	D	C	D	1
										I	B	D	B	D	10
											I	D	D	D	14
												I	D	D	2
													I	D	7
														I	15

I: intermingling

B: bow-tie shaped zone of unstabilized access migration

C: secondary mycelial establishment following B with stabilization

D: deadlock between morphologically distinct mycelia

primary mycelial phases which was widest in non-sib pairings. This was sometimes followed after ca. 7 weeks incubation by the development of white, marginal radial ridges of aerial mycelium within the adjoining regions of primary mycelium. Subculturing revealed that these were zones of further secondary mycelial establishment.

Secondary mycelium developing between pairs of very slowly extending isolates was confined to the central portion of the confrontation zone. If only one slowly extending isolate was present, the secondary mycelium would almost encircle it, but this process was usually halted and a zone of unoccupied medium persisted. Although this was bordered by the mutually inhibited colony margins of both primary and secondary mycelia, there was near-uniformity in the morphology of leading hyphae which were densely packed, narrow, attenuated and characteristic of all relatively slowly extending Stereum primary mycelia.

Pairings which failed to initiate secondary mycelium produced few macroscopic signs of rejection and their constituent isolates remained morphologically distinct (Fig. 4.2c). That they were genetically unaffected by such interactions was borne out by confirmatory testing. Subcultures removed from one side of a confrontation zone intermingled with stock cultures of the isolate originally inoculated on that side of the zone only. Six combinations of isolates allocated to the same mating-type produced $\leq$5mm wide appressed zones, one of which (LQ1-10 x LQ1-7) contained tufts of a resident mycelial type (LQ1-10). Of the remainder, two appression zones failed to produce mycelia which intermingled with one or other of the isolates originally paired, but mycelia were generated that satisfied the adopted criterion for establishment of the secondary phase.

EXPERIMENTAL INTERSPECIFIC PAIRINGS

Interactions between primary mycelia of S. insignitum and S. subtomentosum were all of the rejection type in which the confrontation zone lacked aerial mycelium and was occupied by a relatively wide (≤1cm) region consisting of sparse, frequently disrupted hyphae. This was flanked on either side by a ridge of white or luteous to ochreous aerial mycelium. Subcultures taken from either side of the interaction zone always intermingled with stock cultures of the corresponding isolates, hence there was no evidence for secondary mycelium production and/or access phenomena.

DISCUSSION

Although the collection of S. insignitum spore deposits was very limited and an insufficient basis for generalization, there was a clear demonstration of outcrossing regulated by a unifactorial diaphoromictic system of homogenic incompatibility in this taxon. There was also a propensity for homokaryotic fruiting in a minority of primary mycelia, but this did not preclude override of heterogenic incompatibility by dissimilar mating-type factors.

Rejection zones between LQ1 mating-type incompatible sibs were not as intense as those initially occurring in mating-type compatible pairings between in situ primary and secondary mycelial phases. It seems likely that continued secondary mycelial establishment near the margin of the culture dish, beyond this heterogenic incompatibility barrier, resulted from

nuclear migration within the peripheral region of mated primary mycelia without involving the older central portions. Thus in terms of the adopted working hypothesis outlined in Chapter 1 (pp. 47-52), secondary mycelium is probably established in S. insignitum by the stabilization of access migration. Once this ceases, heterogenic incompatibility is expressed between the secondary and component primary mycelial modes which presumably inhibits heterokaryon/homokaryon mating.

Morphologically, the access reactions of S. insignitum and S. hirsutum were very similar and both terminated in an expression of rejection. An important difference however, is that in S. insignitum access migration was expressed when mating-type compatible combinations were involved, and not epistatically suppressed as in S. hirsutum (Coates et al., 1981). Therefore, it seems likely that control of access migration is a component of the mating-type factors of S. insignitum, although a few exceptional bow-tie reactions occurred between mating-type incompatible sibs (Table 4.1).

This raises the possibility that the bow-tie factor (B-factor) of S. hirsutum could have arisen by duplication of a portion of the mating-type factor which then reinserted elsewhere in the genome. Alternatively, the B-factor could have been a true bifactorial system mating-type factor before duplication and reinsertion in or nearby the present mating-type factor. Hence dissimilarity of B-factors in S. hirsutum merely results in the expression of access migration, whereas dissimilarity of mating-type factors results in stabilized secondary mycelial establishment usually without phenotypic expression of a preliminary access migration stage.

It has been argued that the bifactorial breeding system is the most probable ancestral mating mechanism by virtue of its complexity and wide distribution throughout the homobasidiomycetes (Raper, 1966a; Ullrich & Raper, 1977). However, Raper (1983) recently speculated that both pairs of incompatibility sub-loci which constitute the two mating-type factors of bifactorial primary mycelia probably originated from a single genetic locus. She envisaged that functional divergence of the factors only occurred following duplication of the entire single factor of an ancestral unifactorial form and its subsequent translocation to another chromosome. Another possibility, arising from studies of S. hirsutum, is that the unifactorial mating-type factor is complex (Coates & Rayner, 1985a) and a portion may have been translocated without duplication. This would then explain the characteristic morphogenetic sequences regulated by the A and B factors of bifactorial forms such as S. commune.

Primary mycelium LQ1-13 of S. insignitum was interesting because it seemed to possess a non-parental mating-type factor and would therefore not be subjected to the outbreeding bias imposed on its sibs. Intra-mating-type factor recombination has rarely been demonstrated in unifactorial diaphoromictic forms (see p. 23) and requires the recombination of sub-loci to reform one of the original parental mating-types. A possible alternative method of non-parental factor production involving a disomic mating-type factor was advanced by Flexer (1969) in Polyporus palustris. This was supported by crosses of the anomalous primary mycelia with both parental mating-types which were then fruited and the progeny analysed. Each cross resulted

in approximately equal numbers of progeny bearing the original parental specificities only, hence it was concluded that intra-factor recombination was an unlikely explanation. Furthermore, the same crosses were repeated several weeks later when it was revealed that those isolates previously displaying an anomalous mating pattern had apparently reverted to one of the parental mating-types. The possibility that LQ1-13 was itself a secondary mycelium was rejected because several confirmatory tests of sib pairings revealed the presence of a novel mycelial type within regions originally occupied by LQ1-13, which would not be so if it had become heterokaryotic before the pairing was made. Therefore, intra-factor recombination or disomy remain as possible interpretations of LQ1-13 sib interactions.

Interfertility testing betwen S. insignitum and S. subtomentosum proved inapplicable, because isolates of the latter were all non-outcrossing. Although intermingling of non-sibs was not observed, S. subtomentosum probably has a population structure composed of heterogenically incompatible interaction groups like S. "rameale" and S. sanguinolentum and so the same speculative mode of origin may also apply.

In view of the differences in cultural characteristics and paucity of studied material from the two species, further collections of European material are needed before S. insignitum can be considered as a candidate for the origin of S. subtomentosum by inactivation of the override mechanism. Similarly, the taxonomic validity of the presence or absence of pseudoacanthophyses in distinguishing subgenera within Stereum can only be reviewed after further collections are subjected to experimental pairing tests.

CHAPTER 5 INTRASPECIFIC PRIMARY AND SECONDARY MYCELIAL INTERACTIONS WITHIN A RANGE OF SPECIES

INTRODUCTION

Rayner & Turton (1982) conducted a preliminary investigation of homogenic and heterogenic incompatibility in three collections of S. rugosum which were fruiting on coppiced hazel (C. avellana) in south west England. They concluded that S. rugosum had a unifactorial homogenic I.S. and noted that the production of morphologically distinct secondary mycelium, their criterion for mating-type compatibility, required at least 7 weeks incubation between 15-25°C. A strong heterogenic incompatibility response was evident between paired field isolates from two different decaying hazel poles and a similar result was obtained in some pairings of sib-composed, but not sib-related secondary mycelia. Response intensity was lower in those interactions between secondary mycelia in which all four haploid genotypes were sib-related and was non-existent when pairings were made between subcultures from a single laboratory-synthesized secondary mycelium or between field isolates from a single natural decay column.

This approach was extended in the present investigation of S. rugosum to include two collections from coppiced alder (Alnus) in the Midlands (H1, H2) and one from birch (Betula) in Devon (RT12). Secondary mycelial pairings were performed to include a wide range of nuclear relationships within and between

participating isolates. An investigation was also carried out to detect mating in S. rugosum via the Buller Phenomenon as has been reported for two other British outcrossing members of the genus, i.e. S. hirsutum (Coates et. al., 1981; Coates & Rayner, 1985c) and S. gausapatum (Boddy & Rayner, 1982).

Of the remaining species to be considered in this chapter, results of previous primary mycelial pairings could only be found for P. laevis (Peniophora affinis) reported by Biggs (1938). She concluded that the interactions of 30 sibs conformed to a pattern of bipolar heterothallism. A similar progeny set from a second isolate, identified as Peniophora laevis, only produced clamp connections when in polyspore culture which was taken as an indication of heterothallism. Biggs (1938) also noted multiple clamp connections on hyphae of herbarium specimens of P. velutina (Peniophora velutina) and tissue isolates of C. puteana. However, in the absence of primary mycelia, no conclusions were drawn in respect of their mating systems. C. puteana was also the subject of a cytological investigation by Kemper (1937) who observed the formation of whorled clamp connections on primary mycelia and concluded that sexuality was no longer observable, i.e. C. puteana was not heteromictic.

In view of the existing dearth of information, primary mycelial pairings were set up to confirm Biggs' (1938) finding in P. laevis and to attempt to detect the presence and interplay of incompatibility systems and override in this species, P. velutina and C. puteana. The capacity for interacting conspecific field isolates to show heterogenic incompatibility seems not to have been appreciated in C. puteana, but it has

been used to map population structure of P. velutina in two British woodland sites by Thompson & Boddy (1983).

P. velutina was chosen for an investigation of the relationship between subjectively assessed intensity of heterogenic incompatibility and the genetic relationship of component nuclei to gauge the sensitivity of the reaction and hence its applicability in population studies. The possibility that heterogenic incompatibility could be overridden by the homogenic I.S. leading to secondary mycelial establishment via the Buller Phenomenon was also investigated within P. velutina.

MATERIALS AND METHODS

Tables 2.5-2.8 list the collection sites, codes and numbers of field and primary mycelial isolates of S. rugosum (excluding LG1, GH1, GH2, CH1, CR1, CR2; see Chapter 7), P. velutina, P. laevis and C. puteana that were used in this study. Progeny set members and conspecific field isolates were paired in all their respective combinations and 20-60 intraspecific non-sib combinations were also set up.

Three fruiting primary mycelia of P. laevis and C. puteana were each used to obtain a progeny set of six. Sibs were paired in all combinations and back-paired against their parental isolates.

The same pairing regime was also adopted for three progeny sets of both Phanerochaete spp., each of which was derived from a basidioma produced in sib pairings that satisfied adopted criteria of secondary mycelial development.

Laboratory-synthesized secondary mycelia of S. rugosum and P. velutina were chosen to encompass a wide range of genotypic relationships between their component nuclei and paired in intraspecific combinations and against conspecific field isolates. Similar intraspecific pairings were set up using sib-composed, sib-related secondary mycelia and field isolates of C. puteana and between field isolates of P. laevis.

Intraspecific secondary/primary mycelial pairings were set up between field isolates or laboratory-synthesized secondary mycelia and primary mycelia of S. rugosum and P. velutina. Isolates were also chosen for these interactions such that a wide range of genetic relationships existed between synthesized secondary mycelia and primary mycelia. Similar pairings were set up involving field isolates and progeny of C. puteana and P. laevis. The procedure followed in this study of primary and secondary mycelial interactions is summarized in Fig. 5.1.

Confirmatory testing (see Chapter 2) was applied to the full range of interaction types, including all those which could only be described as weak. The procedure for genotypic analysis of a limited number of secondary/primary mycelial interactions was similar to that of Coates & Rayner (1985c) as shown in Fig. 5.2.

After 10 weeks incubation, a strip of ca. 50 x 5mm was removed from each originally homokaryotic region as shown in Fig. 5.2a and transferred to fresh 2% MA. Resultant zones of rejection between regions of outgrowth from the strip were recorded and a plug of appoximately 5mm diameter was removed from each region. These were transferred to fresh 2% MA in

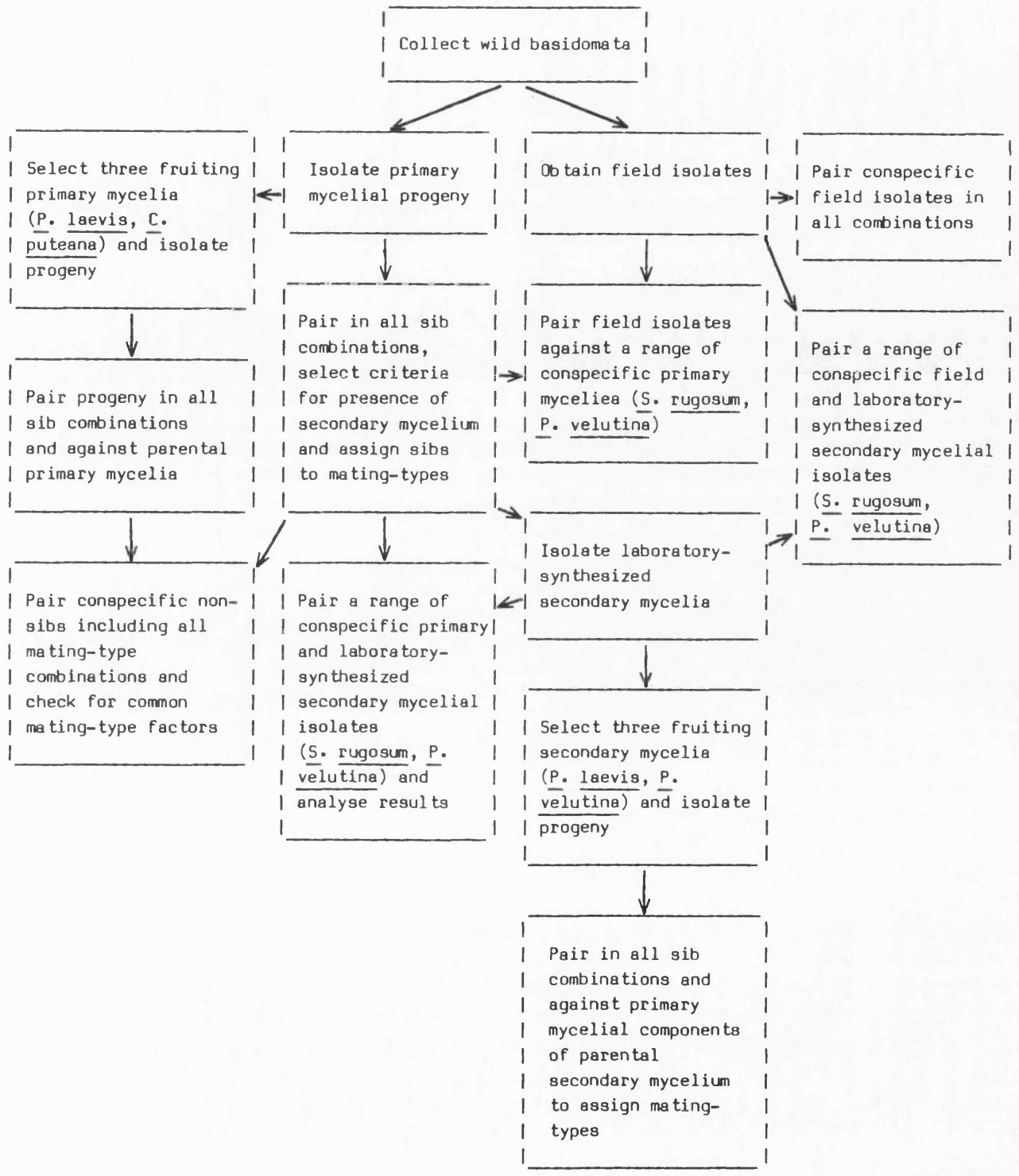


Fig. 5.1. Flowchart summary of procedure for studying primary and secondary mycelial interactions in S. rugosum, P. velutina, P. laevis and C. puteana

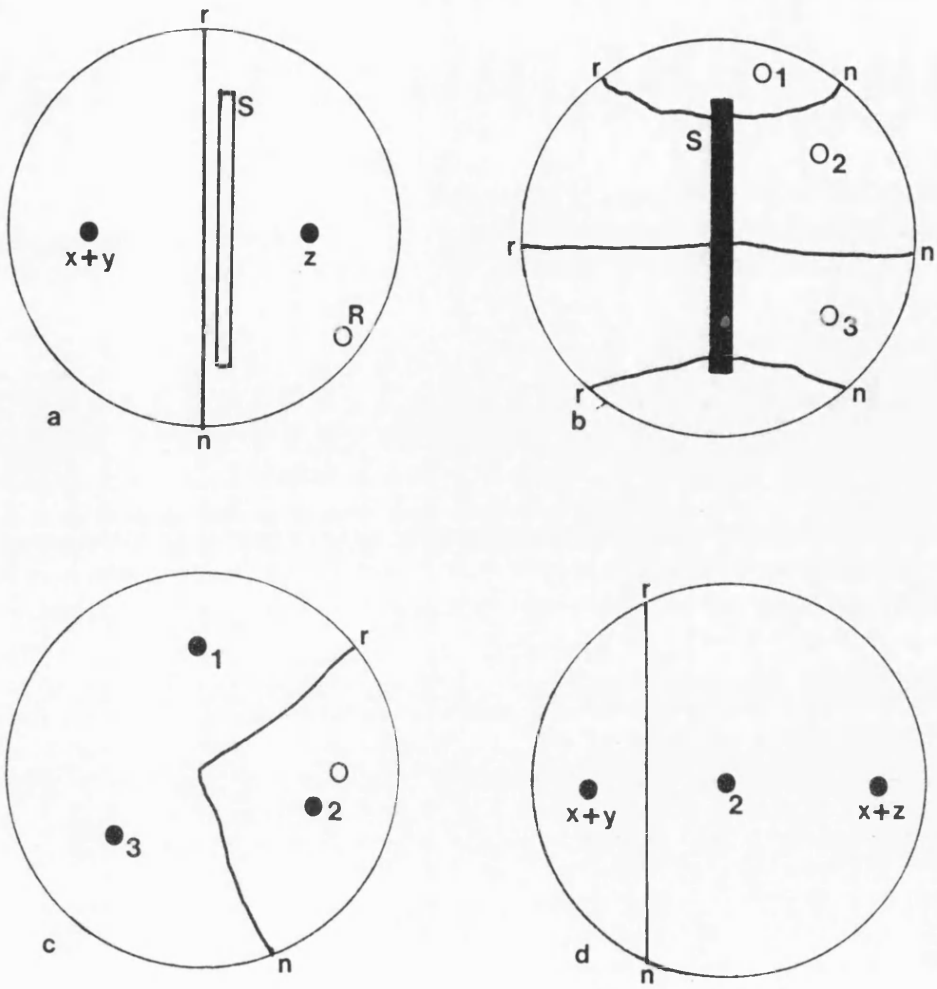


Fig. 5.2. Procedure for analysing the genetic constitution of mycelial types resulting from secondary/primary mycelial interactions. x, y and z represent the three haploid genotypes present, solid circles and box represent inocula, open symbols represent plug removal sites and zones (rn) represent rejection zones. (a) A strip (S) was removed from homokaryon z and placed on fresh medium. (b) After incubation, plugs (1,2,3...) were removed from all regions separated by rejection zones. (c) Trio combinations of subcultures were transferred to fresh medium. After incubation, a single plug was removed from each region separated as before and each (e.g. 2) was placed at the centre of a fresh plate. (d) The genetic constitution of these subcultures was tested against pairs of tester isolates, i.e. homokaryon z and possible heterokaryons of the series x + y, x + z, y + z. Intermingling (e.g. 2 and x + z) was assumed to reveal the identity of the central isolate. A plug (R) was also removed from the original interaction plate (a) remote from the rejection zone and tested as in (d).

combinations of three equidistant plugs per plate. Plugs whose outgrowth subsequently intermingled were assumed to have identical genotypes and once again a plug was removed from each region separated by zones of rejection. These were placed individually at the centre of fresh 2% MA plates and a pair of equidistant tester isolates were inoculated one on either side before incubating for 1 month. Assuming heterogenic incompatibility can be used as a tool to reveal genetic identity under these circumstances, the central isolate could either be assigned to one of the tester mycelial types on the basis of intermingling, or to an unknown type not represented by the tester isolates.

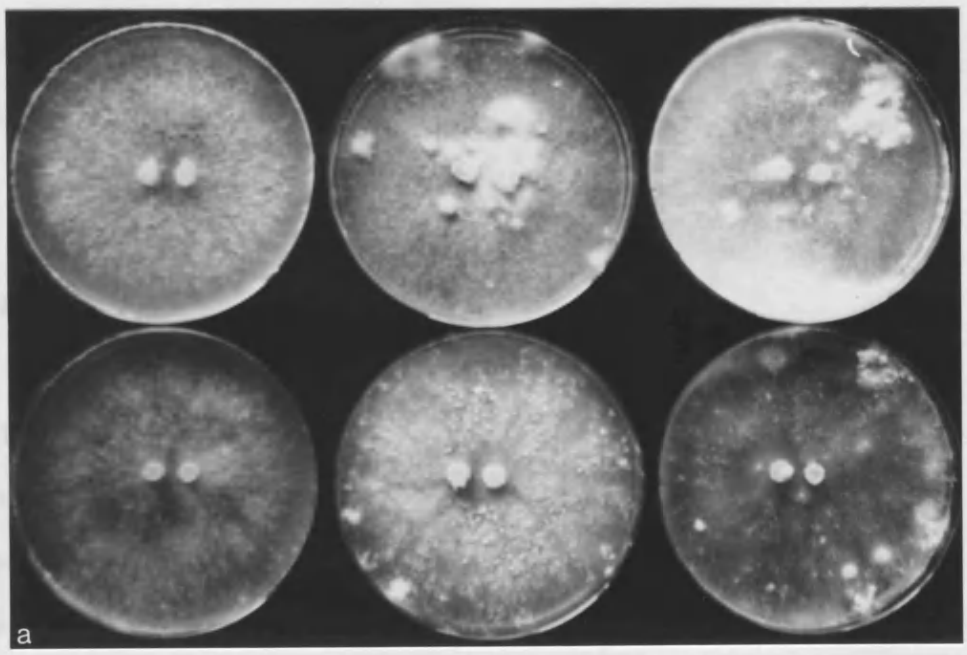
S. rugosum - RESULTS

CULTURAL CHARACTERISTICS

The advancing zone of post-germling mode primary mycelia was appressed, even and bordered a hyaline to white mat which generally had a sparse aerial component. There was some variation within (Fig. 5.3a) and between progeny sets in mycelial density and radial extension rate, but with the exception of two isolates bearing scattered denser superficial white patches, primary mycelia were morphologically relatively similar.

Field isolates were microscopically similar to post-germling mode primary mycelia in having multiple clamp connections on wider hyphae, but differed macroscopically in their development of a cottony to woolly, floccose pale luteous

Fig. 5.3. S. rugosum morphology and mycelial interactions. (a) Range of sib primary mycelial morphology. (b) Non-sib mating-type compatible interaction with hyaline rejection zone (arrowed). (c) Bulked rejection zone between mating-type incompatible sibs. (d) Fully mating-type compatible secondary/primary mycelial interaction showing (left) isolate codes and removal site of strip subculture (S) from within a band of altered mycelial morphology. One boundary of this band is a pigmented rejection zone (R) and the other is arrowed. Component genotypes (right) of secondary mycelia which developed from subculture (S) after removal to fresh medium. (e) Secondary mycelial interactions showing three categories of rejection intensity, strong (left), intermediate (centre) and weak (right).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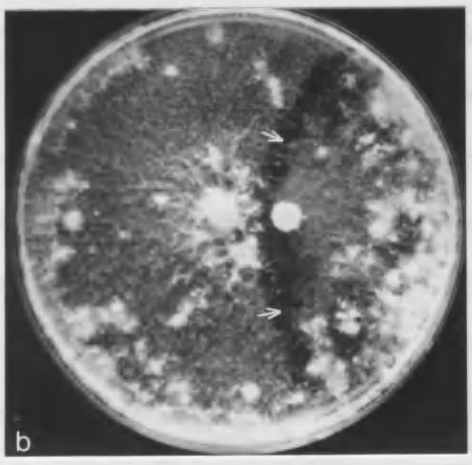
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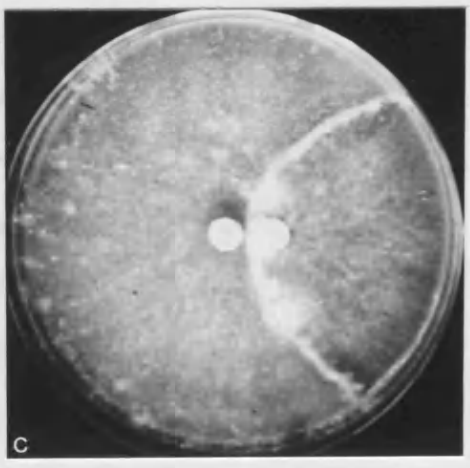
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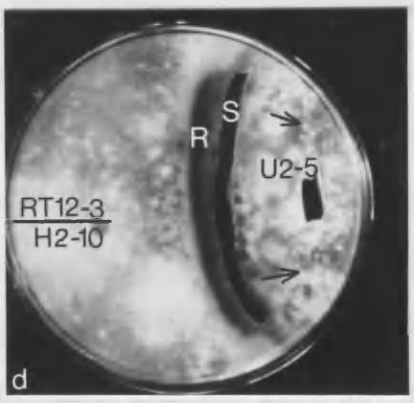
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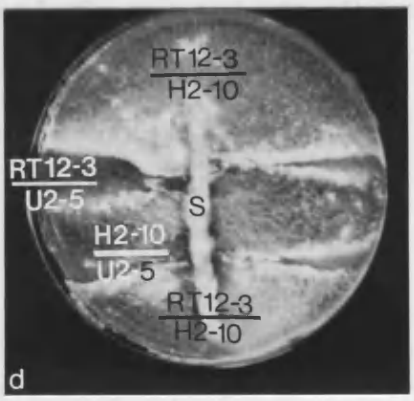
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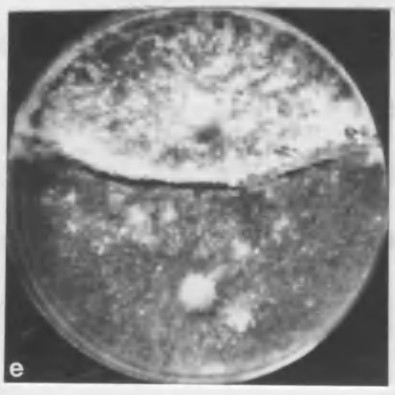
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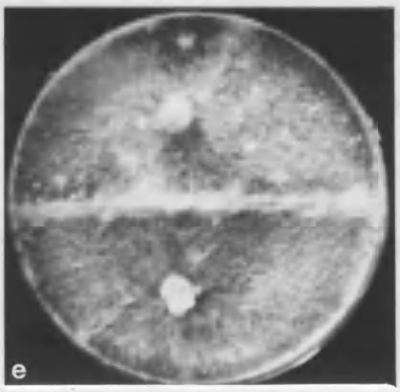
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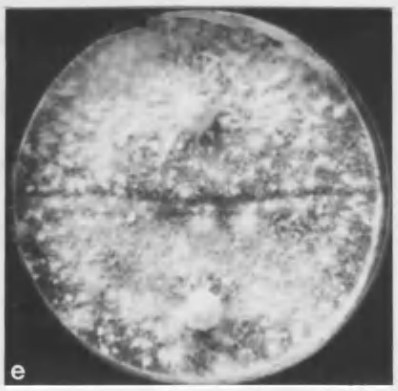
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aerial mycelium in accordance with Rayner & Turton's (1982) observations. No basidiomata of S. rugosum were produced in culture.

EXPERIMENTAL PAIRINGS BETWEEN PRIMARY MYCELIA

The chosen criterion for secondary mycelial establishment was the development of small, dense, white aerial tufts, denser than those on any unpaired primary mycelium, which either merely flanked the confrontation zone, or extended into one or both isolates, rarely becoming confluent. During early stages of secondary mycelial establishment, which could take 3 months before unequivocal tufts were produced, the confrontation zone often remained visible as a hyaline region composed of sparse hyphae and dead compartments. This zone was subsequently obscured in those pairings which resulted in profuse aerial tufts, but otherwise remained distinct, especially in non-sib interactions (Fig. 5.3b). A comparison of the sib and non-sib pairings of particular primary mycelia revealed that the distribution and density of aerial tufts associated with secondary mycelial development was not isolate specific, but seemed dependent on the relationship between the two interacting primary components of each pairing.

Subcultures removed from any site within putative compatible pairings after 3 months incubation yielded intermingling mycelia that were morphologically uniform and distinct from those inoculated. This was interpreted as secondary mycelium and was macroscopically similar to that of cultured field isolates.

In confirmatory tests, secondary mycelium interacted with component primary types to give a narrow lytic confrontation zone flanked by bulked white aerial mycelium with or without underlying orange to sienna pigmentation and intervening exudation of concolorous droplets.

Strip subcultures traversing persistent hyaline confrontation zones of putative mating-type compatible primary mycelia yielded a uniform mycelial morphology and yet a zone of reduced aerial hyphal density was also produced, continuous with and extending <1cm in either direction from that present within the inoculated strip. It seems likely that this was due to further primary mycelial and possibly secondary/primary mycelial interactions which occurred before the colony margin became wholly heterokaryotic.

Pairings which failed to produce tufts also often developed a hyaline interaction zone, although this was delayed relative to those in mating-type compatible pairings. Its development usually followed initial intermingling and sometimes culminated in bulking of aerial mycelium along one or both edges (Fig. 5.3c). Subcultures from such interactions always yielded mycelial types that intermingled with one or other of the originally paired primary mycelial types. Therefore there was no evidence of any genetic exchange leading to altered recognition responses occurring between such deadlocked primary mycelia.

On the basis of the chosen criteria, members of each progeny set were assigned to two mating-type classes indicating a unifactorial homogenic I.S. (Tables 5.1-5.4). Futhermore, all possible combinations of mating-types from the four

Table 5.1. Sib interactions of S. rugosum H1.

Isolate codes															
2	12	15	5	7	4	10	14	6	8	3	13	11	9	1	
I	H	W	H	M	H	H	H	C	C	C	C	C	C	C	2
	I	W	W	W	H	W	W	C	C	C	C	C	C	C	12
		I	W	H	W	W	H	C	C	C	C	C	C	C	15
			I	M	M	H	H	C	C	C	C	C	C	C	5
				I	H	H	W	C	C	C	C	C	C	C	7
					I	H	H	C	C	C	C	C	C	C	4
						I	H	C	C	C	C	C	C	C	10
							I	C	C	C	C	C	C	C	14
								I	W	H	H	M	W	M	6
									I	H	H	H	W	H	8
										I	H	W	H	H	3
											I	H	H	H	13
												I	H	W	11
													I	H	9
														I	1

I: intermingling

C: secondary mycelial establishment

H: hyaline rejection zone

M: mound of bulked aerial mycelium

W: weak interaction

Table 5.2. Sib interactions of S. rugosum H2.

Isolate codes															
2	14	12	11	6	13	15	5	3	1	8	10	9	4	7	
I	M	M	H	H	M	H	H	M	C	C	C	C	C	C	2
	I	M	M	H	M	H	M	M	C	C	C	C	C	C	14
		I	H	H	M	M	H	M	C	C	C	C	C	C	12
			I	H	M	H	M	M	C	C	C	C	C	C	11
				I	H	H	H	M	C	C	C	C	C	C	6
					I	M	H	M	C	C	C	C	C	C	13
						I	H	M	C	C	C	C	C	C	15
							I	M	C	C	C	C	C	C	5
								I	C	C	C	C	C	C	3
									I	H	M	M	M	M	1
										I	M	H	M	M	8
											I	M	M	M	10
												I	M	H	9
													I	H	4
														I	7

I: intermingling

C: secondary mycelial establishment

H: hyaline rejection zone

M: mound of bulked aerial mycelium

Table 5.3. Sib interactions of S. rugosum RT12.

Isolate codes						
1	2	6	5	4	3	
I	M	C	C	C	C	1
	I	C	C	C	C	2
		I	M	M	H	6
			I	H	M	5
				I	M	4
					I	3

I: intermingling
C: secondary mycelial establishment
H: hyaline rejection zone
M: mound of bulked aerial mycelium

Table 5.4. Sib interactions of S. rugosum U2.

Isolate codes						
1	2	3	4	5	6	
I	H	H	H	H	C	1
	I	W	H	H	C	2
		I	H	H	C	3
			I	H	C	4
				I	C	5
					I	6

I: intermingling
C: secondary mycelial establishment
H: hyaline rejection zone
W: weak interaction

basidiomata (H1, H2, RT12, U2) resulted in secondary mycelial establishment indicating a diaphoromictic homogenic I.S.. This also revealed that each mating-type factor was restricted to a single basidioma in the collections studied.

EXPERIMENTAL PAIRINGS BETWEEN SECONDARY MYCELIA

Heterogenic incompatibility was visibly expressed in all 235 pairings of non-identical mycelial types, whereas intermingling was always recorded for control pairings of subcultures from a single stock isolate. Confirmatory testing failed to reveal that any mycelial type was present after an interaction that was not already present in one of the paired inocula.

Rejection intensity was subjectively assigned to three categories as shown in Fig. 5.3e. Strong responses involved the production of a scarlet to orange pigmented zone underlying concolorous droplet exudation from the interface of two bulked ridges of white aerial mycelium. Intermediate responses produced a luteous interaction zone with overlying aerial bulking, but without droplet exudation. Weak responses produced uniformly narrow ($\leq$2mm) zones, lacking pigmentation and flanked by low ridges of white aerial mycelium. These response categories were used to score the outcome of 18 classes of secondary mycelial interaction based on genetic relationship (Table 5.5). Generally, the visible intensity of interaction between paired laboratory-synthesized secondary mycelia diminished with an increase in the relatedness, and hence assumed genetic similarity, of the participating nuclei.

Table 5.5. Subjectively assessed rejection intensity between paired secondary mycelia (heterokaryons) in S. rugosum.

Genetic relationship of nuclear components in paired heterokaryons	Interaction class	Example	Rejection category		
			Strong	Intermediate	Weak
Neither heterokaryon sib-composed and no nuclei sib-related	1	$\frac{U2\text{-}6}{H2\text{-}11} \times \frac{RT12\text{-}6}{H1\text{-}1}$	10	0	0
Neither heterokaryon sib-composed, but one pair of sib-related nuclei of different mating-types	2	$\frac{H1\text{-}2}{H2\text{-}7} \times \frac{H1\text{-}1}{RT12\text{-}5}$	22	0	0
As above, but sib-related nuclei of same mating-type	3	$\frac{H1\text{-}1}{RT12\text{-}5} \times \frac{H1\text{-}11}{H2\text{-}7}$	18	0	4
One heterokaryon sib-composed, but no other sib-related nuclei	4	$U2\ \frac{6}{5} \times \frac{H1\text{-}2}{H2\text{-}7}$	12	0	0
Neither heterokaryon sib-composed, but they have a nucleus in common	5	$\frac{RT12\text{-}4}{H2\text{-}10} \times \frac{RT12\text{-}4}{H1\text{-}2}$	11	2	9
As above with two pairs of sib-related nuclei of different mating-types	6	$\frac{RT12\text{-}1}{H2\text{-}7} \times \frac{RT12\text{-}5}{H2\text{-}11}$	7	4	1
As above, but with one pair of sib-related nuclei of different mating-type	7	$\frac{U2\text{-}4}{RT12\text{-}6} \times \frac{U2\text{-}5}{RT12\text{-}1}$	7	4	1
As above, but with two pairs of sib-related nuclei of same mating-type	8	$\frac{RT12\text{-}3}{H2\text{-}10} \times \frac{RT12\text{-}5}{H2\text{-}7}$	7	5	0

Table 5.5. (cont.).

Genetic relationship of nuclear components in paired heterokaryons	Interaction class	Example	Rejection category		
			Strong	Intermediate	Weak
Both heterokaryons sib-composed but not sib-related	9	H1 $\frac{7}{13}$ x H2 $\frac{6}{8}$	12	0	0
Neither heterokaryon sib-composed, but one common nucleus and one pair of sib-related nuclei of different mating-types	10	$\frac{\text{RT12-6}}{\text{H1-2}}$ x $\frac{\text{RT12-6}}{\text{H1-1}}$	3	3	6
As above, but sib-related nuclei of same mating-type	11	$\frac{\text{U2-6}}{\text{H2-11}}$ x $\frac{\text{U2-6}}{\text{H2-14}}$	4	5	3
One heterokaryon sib-composed and one pair of sib-related nuclei	12	H2 $\frac{6}{8}$ x $\frac{\text{H2-11}}{\text{RT12-5}}$	11	1	0
As above, but one pair of nuclei common	13	H1 $\frac{2}{1}$ x $\frac{\text{H1-1}}{\text{RT12-6}}$	10	2	0
Both heterokaryons sib-composed and sib-related, but no nuclei in common	14	H2 $\frac{12}{8}$ x $\frac{3}{1}$	5	1	6
As above with one nucleus in common	15	U2 $\frac{6}{1}$ x $\frac{6}{5}$	0	4	5
Two field isolates	16	ROlw x Qlw	6	0	0
One non-sib-composed heterokaryon and unrelated field isolate	17	$\frac{\text{RT12-6}}{\text{H2-10}}$ x Tlw	11	1	0

EXPERIMENTAL PAIRINGS BETWEEN SECONDARY AND PRIMARY MYCELIA

Heterogenic incompatibility was visibly expressed in all 98 pairings of non-identical mycelial types. Interaction intensity was subjectively assigned to the three categories, i.e. strong, intermediate and weak, as applied to strictly secondary mycelial interactions. Table 5.6 shows the results of eight classes of secondary/primary mycelial pairings based on the genetic relationship of their constituent genotypes. Generally, the visible intensity of interaction diminished with an increase in the inferred genetic similarity of the participating nuclei as occurred between different secondary mycelia.

Pairings in which the primary mycelial genotype was absent from the secondary mycelium developed a band of altered morphology within the original homokaryon immediately adjacent to the confrontation zone (Fig. 5.3d). This became macroscopically mottled and, when viewed through the base of the dish, was sometimes irregularly pigmented orange to scarlet. Microscopically, this band contained hyphae with scattered regions of prolific branching and congealed orange to scarlet contents. Strip subcultures from this region sometimes generated similarly flat and mottled mycelium, but further subculturing increased aerial mycelium production and branching became more regular. Thereafter, such mycelia always intermingled with one of the mycelial types already generated by the same strip subculture. When the primary mycelial genotype was also present within the secondary mycelium, the mottled band

Table 5.6. Subjectively assessed rejection intensity in *S. rugosum* secondary/primary mycelial (heterokaryon/homokaryon) pairings.

Genetic relationship of nuclear components in heterokaryon/homokaryon pairings	Interaction class	Example	Rejection category		
			Strong	Intermediate	Weak
Heterokaryon non-sib-composed and homokaryon unrelated	1	$\frac{\text{RT12-4}}{\text{H1-2}}$ x U2-5	17	1	0
Heterokaryon sib-composed and homokaryon non-sib-related	2	U2 $\frac{6}{5}$ x H1-1	12	0	0
Heterokaryon non-sib-composed, homokaryon sib-related and of different mating-type	3	$\frac{\text{H1-5}}{\text{H2-7}}$ x H2-14	11	1	0
As above, but homokaryon of same mating-type	4	$\frac{\text{RT12-3}}{\text{H2-10}}$ x RT12-5	8	2	2
As above, but one nucleus in common	5	$\frac{\text{H1-5}}{\text{H2-7}}$ x H1-5	5	3	4
Heterokaryon sib-composed and sib-related to homokaryon	6	H2 $\frac{3}{1}$ x H2-10	1	4	7
As above, but one nucleus in common	7	H1 $\frac{2}{1}$ x H1-1	1	4	3
Field isolate unrelated to homokaryon	8	Q1w x RT12-1	12	0	0

was inconspicuous and developing aerial mycelium soon became confluent on either side of and obscured the confronation zone.

Results of 35 interactions subjected to genotypic analysis are shown in Table 5.7. A trio of sibs was involved in the only interaction which failed to produce detectable heterokaryosis within the incumbent homokaryon. Of the remainder, 27 pairings (classes 1-4 and 6) could theoretically have resulted in the production of at least one detectable composite heterokaryon, i.e. containing the nuclear component of the resident homokaryon together with one of those present in the resident heterokaryon. The results of 25 such pairings conformed to this prediction and of these the recovery of but a single heterokaryon was the most frequent outcome (80%). Combinations of parental and composite heterokaryons were only detected within fully mating-type compatible pairings (classes 1-3), two of which involved the recovery of three heterokaryons from the formerly homokaryotic region (Fig. 5.3d). Inoculated heterokaryons were the only type recovered from formerly homokaryotic regions of the two interactions, both involving trios of sibs, which failed to produce a theoretically possible composite, and also from all those in which a haploid genotype was common to both inocula.

Interaction class 3 was particularly interesting because both haploid genotypes within the heterokaryon were mating-type compatible with that of the homokaryon. Moreover, one possible composite heterokaryotic combination would be sib-composed, whereas the other would be non-sib-composed. Therefore, this class permits an investigation of preferential non-sib or sib selection during heterokaryosis (see pp.41-43). Table 5.8 shows

Table 5.7. Genotypic analysis of S. rugosum secondary/primary mycelial pairings.

Interaction class (see Table 5.6)	Number of interactions analysed	Number and identity of heterokaryotic genotypes detected in formerly homokaryotic regions					Number of interactions in which the inoculated homokaryon was produced by plug subculturing the periphery of the formerly homokaryotic region
		ONE		TWO		THREE	
		Inoculated type only	Composite type only	Inoculated and one composite type	Both composite types	Inoculated and two composite types	
1	4	0	2	1	0	1	0
2	3	0	2	0	0	1	1
3	7	0	5	2	0	0	0
4	7	0	7	0	0	0	1
6	7	2	4	0	0	0	3
5+7	7	7	0	0	0	0	1

Table 5.8. Sib-related and non-sib-related nuclear selection in *S. rugosum* secondary/primary mycelial pairings (interaction class 3, see Table 5.6).

Identity of heterokaryon in pairing	Identity of homokaryon in pairing					
	H2-11	H2-8	H2-14	H1-1	H1-5	H1-11
RT12-3 / H2-10	non-sib selection	---	---	---	---	---
H2-6 / U2-6	---	non-sib selection	---	---	---	---
U2-6 / H2-14	---	non-sib selection	---	---	---	---
U2-6 / H2-8	---	---	non-sib selection	---	---	---
RT12-4 / H1-2	---	---	---	non-sib selection	---	---
U2-4 / H1-11	---	---	---	---	sib* selection	---
U2-4 / H1-5	---	---	---	---	---	non-sib* selection

Note: results marked thus* involved the same trio of primary mycelial genotypes

that of all seven pairings resulting in the establishment of a composite heterokaryon, only one was the product of preferential sib selection. Of the remaining non-sib selected heterokaryons, one was the product of an interaction between the same three genotypes that had resulted in the exceptional case of sib selection. This suggests that selection may operate on both heterokaryotic nuclear dissociation and association processes.

P. velutina - RESULTS

CULTURAL CHARACTERISTICS

The equivalent of a germling mode as applied within Stereum (see p.107) was characterized in P. velutina by verrucose clampless hyphae which repeatedly grew in curves and spirals as if attracted to older portions of the mycelium. This mode was superseded by radially orientated growth of smoother-walled hyphae which produced an appressed primary mycelium with a macroscopically fimbriate margin of distant hyphae. Nevertheless, established primary mycelia exhibited a wide range of morphology, even within progeny sets (Fig. 5.4a). Once the edge of the Petri dish had been reached, the peripheral mycelial zone became white and floccose to plumose surrounding a downy cottony central region. Alternatively, the central mat developed superficial and submerged silky cord-like lengths, interspersed with autolytic regions, and truly aerial mycelium was restricted to vertical cords around the colony margin. Most isolates were hyaline to white, however patches of fulvous to chestnut became prominent in several cases. These patches were

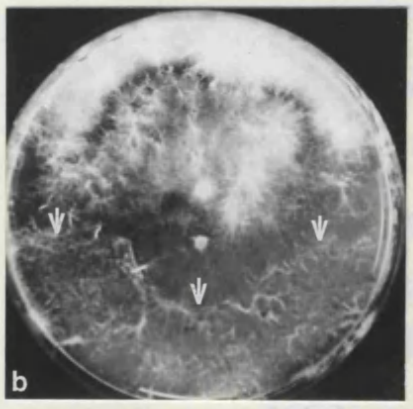
Fig. 5.4. P. velutina morphology and mycelial interactions. (a) Range of sib primary mycelial morphology. (b,c) Sib mating-type compatible pairings showing secondary mycelial establishment localized within a lytic region which in (b) occurs throughout the upper half of the dish and extends to the arrows, leaving a remnant of the resident primary mycelium beyond, and in (c) occupies the entire dish excepting those regions (H) containing pigmented and "blotchy" remnants of the resident U5 primary mycelium. (d) Sib mating-type incompatible pairing. (e-g) Secondary mycelial interactions showing two categories of rejection intensity, (e) weak and (f,g) strong. (g) Fan-shaped aerial mycelial invasion (arrowed). (h,i) Secondary/primary mycelial pairings showing (h) strong rejection and (i) resident secondary mycelial invasion of primary mycelium (arrowed). (j) Mycelium generated by plug (Y), removed from formerly homokaryotic region of

$$F13\ \frac{12}{7} \times F27\text{-}14,$$

intermingling with that of $\frac{F13\text{-}12}{F27\text{-}14}$, indicating probable genetic identity, but weakly rejecting (R) $\frac{F13\text{-}7.}{F27\text{-}14}$

The latter is an alternative composite heterokaryon that could theoretically have been generated by the subcultured interaction via the Buller Phenomenon.


a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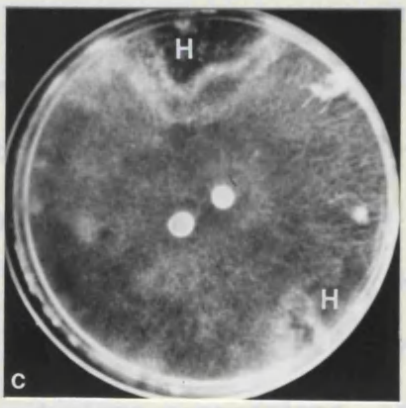
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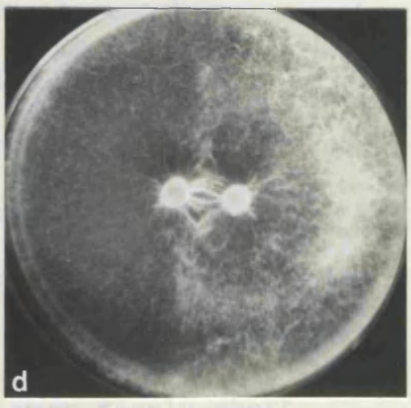
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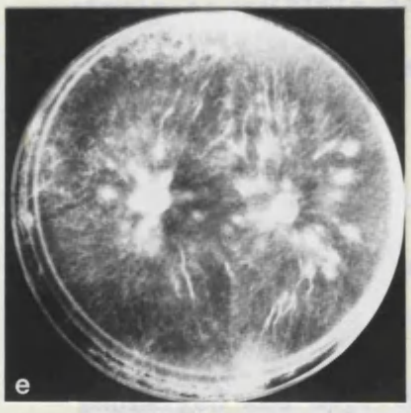
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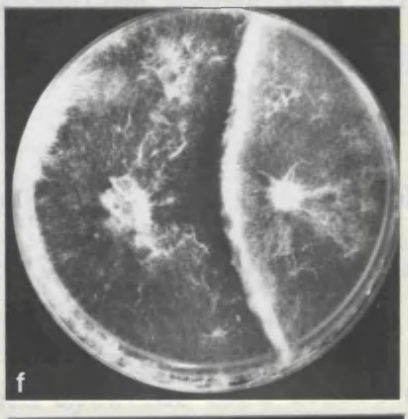
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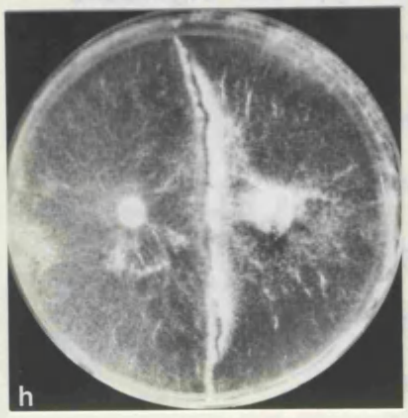
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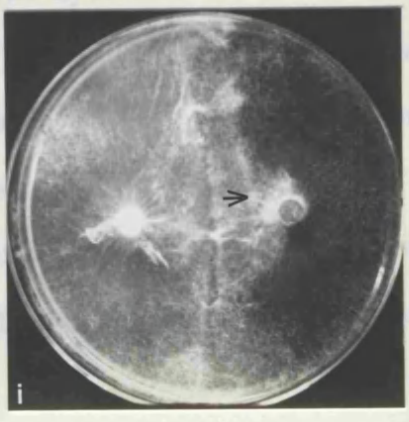
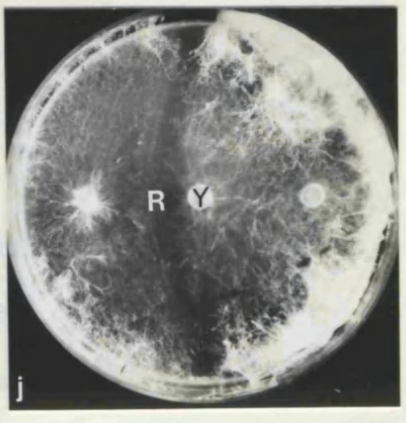

i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j

associated with lengths of concolorous congealed protoplasm and may indicate constitutive instability.

Microscopically, the mycelia were composed of frequently-anastomosed relatively wide hyphae ($\leq 14\mu m$) whose advancing front characteristically contained elements showing sympodial proliferation. Older wide hyphae were frequently encrusted with crystals, probably of calcium oxalate as determined by Thompson & Rayner (1983) for similarly encrusted outer layers of excavated mycelial cords. Hyphal branches occasionally comprised short, narrow monilioid compartments or contrastingly swollen globose compartments and balloon-like regions of wall deformation (c.f. Burdsall & Gilbertson, 1974). Approximately 50% of the isolates contained dense patches of interwoven straight hyphae with numerous constricted septa and short interconnecting hyphae (H-bridges). When observed under phase-contrast optics, some septa were clearly associated with single or multiple pseudoclamp connections, or very rarely short true clamp connections. The latter could occur singly or within a pseudoclamp whorl.

Field isolates differed from established primary mycelia in their often higher radial extension rate, greater proportion of straight, clamped sparsely-branched hyphae and propensity for submerged and appressed mycelial cord formation. The mats were also relatively less dense, hyaline to white and produced scanty downy aerial mycelium which was aggregated into vertical cords on the side of the Petri dish.

Laboratory-produced basidiomata resembled those occurring in the field, but were only produced by field isolates or in certain paired combinations of primary mycelia.

Basidiospores could be collected after 4 weeks dark incubation at 20°C followed by 3 weeks ambient (15-25°C) incubation on the laboratory bench.

EXPERIMENTAL PAIRINGS BETWEEN PRIMARY MYCELIA

The chosen criterion for secondary mycelial establishment was the development of a central macroscopically lytic region containing hyphae with whorled clamp connections bordered by emergent mycelial cords with the microscopic characteristics of field isolates (Fig. 5.4b,c). The extent of lysis varied both between dishes and between members of a pairing. Some isolates consistently showed a relatively small lytic area regardless of the identity of the opposing isolate, but in the majority its extent seemed dependent on the particular combination present.

In examples where the lytic region failed to occupy the entirety of the dish, marginal regions remained whose morphology suggested they were remnants of original primary mycelia and this was borne out by confirmatory testing. Subcultures from lytic regions always gave rise to a mycelium with field isolate characteristics which failed to intermingle with either component primary isolate. By contrast, plugs taken from suspected primary remnants always intermingled with the inoculated mycelial type, thus confirming their status as primary mycelial refugia. Secondary mycelial tufts and cords emanating from the edge of the lytic region overarched this boundary and returned to the culture surface within the refugia. This was followed by a fulvous-chestnut pigmented reaction which

mimicked that of lytic region subcultures when confronted with component primary mycelia. Extension of these cords was subsequently halted although whole refugia sometimes acquired concolorous pigmentation.

Mating-type compatible sibs from U5 produced marginal areas resembling primary refugia, but in which there was also an accompanying morphological change. These regions were strikingly mottled or "blotchy" (Fig. 5.4c) and microscopically comprised widely separated regions of densely branched hyphae and also ghosts of secondary hyphae. On subculture, secondary mycelium emerged which intermingled with that derived from lytic regions. A similar situation obtained in non-sib pairings of an F13 derived primary mycelium against members of one U5 mating-type class. The former became macroscopically lytic throughout and secondary mycelium was very sparsely distributed therein, whereas the latter contained relatively dense patches of the secondary phase. Microscopically, the F13 side of these pairings comprised hyphae whose morphology resembled that of mottled mycelium. Subculturing from this region yielded recognizable secondary mycelium which intermingled with that derived from the U5 side of the pairing.

Weakly interacting putatively mating-type compatible primary mycelia yielded the secondary phase when subcultures were removed from the confrontation zone itself. A single rust coloured "blocker" primary mycelium (IN1-2) was isolated which enabled secondary mycelium to develop from mating-type compatible primary mycelia against which it was paired, but not from itself. Plugs of "blocker" mycelium subcultured from such pairings always intermingled with corresponding stock cultures

indicating their inability to reciprocate in establishment of the secondary phase. In most pairings against mating-type incompatible sibs, the "blocker" was obscured by overarching aerial mycelium from the other member of the pairing.

Two mating-type classes were found within each of the six sets of sibs indicating a unifactorial homogenic I.S. (Tables 5.9-5.14). The larger sets showed an imbalance in recovery frequency for the two mating-types of approximately 2:1 in F25, F13, F27 and 4:1 in U5 where mating-type compatibility was reduced to approximately 33%.

The majority of mating-type incompatible sib interactions were recognizable by the absence of lysis and secondary mycelial establishment, rather than by a macroscopic expression of heterogenic incompatibility *per se* (Fig. 5.4d). Narrow (1-2mm) hyaline interaction zones were infrequently observed and pigmented zones were restricted to 11 pairings of isolates within progeny sets F25 and F13. These sets also exhibited the greatest lytic intensity preceding secondary mycelial development. Mating-type incompatible interactions were also observed which had some, but not all, features of mating-type compatibility, i.e. areas of lysis and/or arching aerial cord formation without regular whorled clamp connections, but their occurrence within a single progeny set lacked any obvious pattern.

All non-sib interactions resulted in secondary mycelial establishment, indicating that *P. velutina* is diaphoromictic and each mating-type factor was represented once only in the collection of wild basidiomata.

Table 5.9. Sib interactions of P. velutina F25.

								Isolate codes											
5	15	4	18	8	16	20	10	2	11	12	13	7	19	17	1	6	9	3	14	
I	L	L	P	M	L	L	P	L	L	M	L	L	C	C	C	C	C	C	C	5
	I	D	L	M	D	D	D	D	L	M	M	D	C	C	C	C	C	C	C	15
		I	M	M	L	M	P	D	L	M	L	L	C	C	C	C	C	C	C	4
			I	M	P	M	L	W	M	M	M	D	C	C	C	C	C	C	C	18
				I	M	M	D	D	L	P	L	L	C	C	C	C	C	C	C	8
					I	P	D	D	P	M	D	P	C	C	C	C	C	C	W	16
						I	M	W	M	W	L	D	C	C	C	C	C	C	C	20
							I	D	L	M	L	M	C	C	C	C	C	C	C	10
								I	M	D	M	P	C	C	C	C	C	C	C	2
									I	D	L	L	C	C	C	C	C	C	C	11
										I	L	L	C	C	C	C	C	C	C	12
											I	P	C	C	C	C	C	C	C	13
												I	C	C	C	C	C	C	C	7
													I	D	L	M	D	D	W	19
														I	D	D	D	D	L	17
															I	D	L	M	D	1
																I	L	D	D	6
																	I	D	H	9
																		I	D	3
																			I	14

I: intermingling

L: central lytic region without secondary mycelial establishment

C: secondary mycelial establishment within L

D: deadlock between morphologically distinct mycelia

H: hyaline rejection zone

P: pigmented rejection zone

M: mound of bulked aerial mycelium

W: weak interaction

Table 5.10. Sib interactions of P. velutina F13.

	Isolate codes																			
	6	8	13	5	17	19	14	1	2	15	11	12	16	20	3	9	18	4	7	10
6	I	D	M	M	W	W	W	W	D	D	D	W	W	C	C	C	C	C	C	C
8		I	M	M	W	M	H	M	L	W	W	W	W	C	C	C	C	C	C	C
13			I	M	L	M	W	M	M	M	M	L	D	C	C	C	C	C	C	C
5				I	D	M	W	W	M	W	M	W	M	C	C	C	C	C	C	C
17					I	L	L	W	D	W	W	D	W	C	C	C	C	C	W	C
19						I	P	W	D	M	W	H	W	C	C	C	C	C	C	C
14							I	W	M	W	D	W	W	C	C	C	C	C	C	C
1								I	M	M	D	W	W	C	C	C	C	C	C	C
2									I	M	W	W	L	C	C	C	C	C	C	C
15										I	W	L	W	C	C	C	C	C	C	C
11											I	H	W	C	C	C	C	C	C	C
12												I	D	C	C	C	C	C	C	C
16													I	C	C	C	C	C	C	C
20														I	M	W	L	L	W	W
3															I	M	L	L	H	L
9																I	L	L	H	W
18																	I	L	L	L
4																		I	L	L
7																			I	D
10																				I

I: intermingling

L: central lytic region without secondary mycelial establishment

C: secondary mycelial establishment

D: deadlock between morphologically distinct mycelia

H: hyaline rejection zone

P: pigmented rejection zone

M: mound of bulked aerial mycelium

W: weak interaction

Table 5.11. Sib interactions of *P. velutina* F27.

	Isolate codes												
13	9	2	5	1	8	11	10	12	14	3	15	7	
I	M	M	W	W	W	H	M	H	W	W	W	C	13
	I	M	W	M	W	W	W	W	C	C	C	C	9
		I	H	W	W	W	W	W	W	W	W	C	2
			I	W	W	W	W	W	W	W	W	C	5
				I	W	W	M	M	C	C	C	C	1
					I	W	W	W	W	W	W	C	8
						I	W	W	W	W	W	C	11
							I	W	C	C	C	C	10
								I	W	W	W	C	12
									I	H	W	W	14
										I	H	W	3
											I	M	15
												I	7

I: intermingling
C: secondary mycelial establishment
H: hyaline rejection zone
M: mound of bulked aerial mycelium
W: weak interaction

Table 5.12. Sib interactions of *P. velutina* U5.

	Isolate codes																		
13	17	18	10	14	7	1	3	16	12	6	8	11	2	5	20	19	15	9	4	
I	W	M	W	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	13
	I	W	W	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	17
		I	W	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	18
			I	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	10
				I	W	W	W	W	M	M	M	W	M	M	W	W	W	M	W	14
					I	W	M	W	W	M	W	W	M	W	W	M	W	W	W	7
						I	W	W	L	W	W	W	M	W	M	W	M	W	W	1
							I	W	M	M	M	W	W	W	M	W	W	M	W	3
								I	W	W	W	W	M	L	W	M	M	W	W	16
									I	M	L	W	M	W	W	M	W	M	W	12
										I	M	W	M	M	M	M	W	W	W	6
											I	W	M	W	W	W	W	W	W	8
												I	M	W	M	W	W	W	W	11
													I	W	M	M	M	M	W	2
														I	W	M	W	M	W	5
															I	M	W	L	W	20
																I	M	M	W	19
																	I	L	W	15
																		I	W	9
																			I	4

I: intermingling

L: central lytic region without secondary mycelial establishment

C: secondary mycelial establishment

M: mound of bulked aerial mycelium

W: weak interaction

Table 5.13. Sib interactions of *P. velutina* IN1.

	Isolate codes							
7	6	2	3	1	4	8	5	
I	H	D	D	M	C	C	C	7
	I	M	L	M	C	C	C	6
		I	D	M	C	C	W	2
			I	L	C	C	C	3
				I	C	C	C	1
					I	M	D	4
						I	D	8
							I	5

I: intermingling
L: central lytic region without secondary mycelial establishment
C: secondary mycelial establishment
D: deadlock between morphologically distinct mycelia
H: hyaline rejection zone

Table 5.14. Sib interactions of *P. velutina* G1.

	Isolate codes							
6	8	2	3	1	7	4	5	
I	L	C	C	C	C	C	C	6
	I	C	C	C	C	C	C	8
		I	H	H	W	W	H	2
			I	H	L	W	W	3
				I	D	L	L	1
					I	W	H	7
						I	L	4
							I	5

I: intermingling
L: central lytic region without secondary mycelial establishment
C: secondary mycelial establishment
D: deadlock between morphologically distinct mycelia
H: hyaline rejection zone
W: weak interaction

Sibs within progeny sets from laboratory-synthesized secondary mycelia interacted according to the unifactorial pattern, but failed to produce basidiomata. Microscopically, their mating-type compatible interactions seemed to be generally weaker and associated with less lysis and secondary mycelial emergence than was evident in sib pairings of their parental isolates. Members of each mating-type class were mating-type compatible with one of the parental mycelia and remained slightly morphologically distinct from the other in the absence of secondary mycelial development. The two mating-types in the progeny sets were therefore identical to those of parental mycelia.

EXPERIMENTAL PAIRINGS BETWEEN SECONDARY MYCELIA

Twenty confrontations of the 349 inspected resulted in intermingling which was indistinguishable from control pairings which should be borne in mind when considering analyses of natural population structure. All other interactions resulted in an expression of heterogenic incompatibility, but in no case did confirmatory tests reveal a mycelial type that was not an inoculated type.

Two subjectively assessed response categories were used in scoring secondary mycelial interactions as follows. Strong responses (Fig. 5.4f,g) involved a hyaline lytic zone (2-4mm wide) and/or white aerial bulking of mycelium with underlying sienna to chestnut pigmentation usually with a pronounced bay component. Ridges of aerial mycelium sometimes produced fan-shaped mycelial sheets which overarched the confrontation zone

and penetrated the mycelium beyond (Fig. 5.4g). These were usually arrested within 1cm of their inception associated with surrounding bay or chestnut pigmentation. Rarely, however, the sequence continued with the apparent senescence of the aerial fans, rendering then concolorous with the underlying medium. These regions soon became reoccupied by aerial mycelium, but this time it was of resident, not replacing, mycelial type. Weak responses (Fig. 5.4e) involved a narrower ($\leq$2mm) hyaline zone of lysis which lacked flanking regions of bulked aerial mycelium and became indistinct at the edge of the Petri dish.

These response categories were used to score the outcome of 20 classes of secondary mycelial interaction based on genetic relationship (Table 5.15). Generally, the visible intensity of interaction between laboratory-synthesized mycelia diminished with an increase in the relatedness, and hence assumed genetic similarity of the participating nuclei.

EXPERIMENTAL PAIRINGS BETWEEN SECONDARY AND PRIMARY MYCELIA

Heterogenic incompatibility was visibly expressed (Fig. 5.4h) in 157 of 163 inspected pairings, whereas the remainder intermingled as control pairings. Interaction intensity was subjectively assigned to the three categories, i.e. strong, weak and intermingling, as applied to strictly secondary mycelial interactions. Table 5.16 shows the results of nine classes of secondary/primary mycelial pairings based on the genetic relationship of their constituent genotypes. Generally, the visible intensity of interaction diminished with an increase in

Table 5.15. Subjectively assessed rejection intensity between paired secondary mycelia (heterokaryons) in P. velutina.

Genetic relationship of nuclear components in paired heterokaryons	Interaction class	Example	Rejection category		
			Strong	Weak	Intermingling
Neither heterokaryon sib-composed and no nuclei sib-related	1	U5-10 / F25-15 x F13-7 / F27-14	11	1	0
Neither heterokaryon sib-composed, but one pair of sib-related nuclei of different mating-types	2	F27-10 / F25-3 x F27-14 / U5-20	11	1	0
As above, but sib-related nuclei of same mating-type	3	F27-7 / F13-6 x F27-14 / U5-20	11	1	0
One heterokaryon sib-composed, but no other sib-related nuclei	4	F27 14/1 x F25-9 / F13-2	15	0	0
Neither heterokaryon sib-composed, but they have a nucleus in common	5	F13-6 / F27-7 x F13-6 / F25-9	10	2	0
As above with two pairs of sib-related nuclei of different mating types	6	F25-3 / F13-19 x F25-11 / F13-18	12	4	1
As above, but with one pair of sib-related nuclei of different mating-type	7	F25-11 / F13-18 x F25-15 / F13-6	12	3	2
As above, but with two pairs of sib-related nuclei of same mating-type	8	F25-11 / F13-18 x F25-15 / F13-7	13	4	0
Both heterokaryons sib-composed but not sib-related	9	F25 19/13 x F13 16/3	18	0	0

Table 5.15. (Cont.)

Genetic relationship of nuclear components in paired heterokaryons	Interaction class	Example	Rejection category		
			Strong	Weak	Intermingling
Neither heterokaryon sib-composed, but one common nucleus and one pair of sib-related nuclei of different mating-types	10	$\frac{F27\text{-}1}{F13\text{-}18} \times \frac{F27\text{-}1}{F13\text{-}2}$	6	6	4
As above, but sib-related nuclei of same mating-type	11	$\frac{F25\text{-}3}{F13\text{-}19} \times \frac{F25\text{-}3}{F13\text{-}2}$	12	3	2
One heterokaryon sib-composed and one pair of sib-related nuclei	12	$\frac{F13\text{-}6}{F27\text{-}7} \times F27\ \frac{14}{1}$	17	2	0
As above, but one pair of nuclei common	13	$\frac{F13\text{-}6}{F27\text{-}14} \times F27\ \frac{1}{14}$	16	3	0
Both heterokaryons sib-composed and sib-related, but no nuclei in common	14	$U5\ \frac{4}{18} \times \frac{14}{10}$	10	5	4
As above, with one nucleus in common	15	$F13\ \frac{1}{20} \times \frac{15}{20}$	8	5	6
Two field isolates	16	F10w x Y4w	36	0	0
One non-sib-composed heterokaryon and unrelated field isolate	17	$\frac{F13\text{-}6}{F27\text{-}14}$ x Y8w	18	0	0
One heterokaryon sib-composed and unrelated field isolate	18	$F25\ \frac{14}{16} \times$ Y3w	24	0	0

Table 5.15. (Cont.)

Genetic relationship of nuclear components in paired heterokaryons	Interaction class	Example	Rejection category		
			Strong	Weak	Intermingling
Non-sib-composed heterokaryon and field isolate with one parental nuclear type	19	$\frac{\text{F13-6}}{\text{U5-20}}$ x U5w	18	0	0
Sib-composed heterokaryon and parental field isolate	20	F13 $\frac{20}{1}$ x F13w	8	3	1

Table 5.16. Subjectively assessed rejection intensity in *P. velutina* secondary/primary mycelial (heterokaryon/homokaryon) pairings.

Genetic relationship of nuclear components heterokaryon/homokaryon pairings	Interaction class	Example	Rejection category		
			Strong	Weak	Intermingling
Heterokaryon non-sib-composed and homokaryon unrelated	1	$\frac{\text{F13-6}}{\text{F27-7}}$ x U5-10	18	0	0
Heterokaryon sib-composed and homokaryon non-sib-related	2	F27 $\frac{15}{1}$ x F25-9	17	1	0
Heterokaryon non-sib-composed, homokaryon sib-related and of different mating-type	3	$\frac{\text{F27-14}}{\text{U5-20}}$ x U5-10	16	2	0
As above, but homokaryon of same mating-type	4	$\frac{\text{F13-6}}{\text{F27-14}}$ x F13-19	14	3	1
As above, but one nucleus in common	5	$\frac{\text{F13-6}}{\text{F27-7}}$ x F13-6	12	5	1
Heterokaryon sib-composed and sib-related to homokaryon	6	F27 $\frac{3}{2}$ x F27-14	8	2	1
As above, but one nucleus in common	7	F25 $\frac{2}{14}$ x F25-2	5	4	3
Field isolate unrelated to homokaryon	8	Y3w x F25-3	35	0	0
Field isolate and related homokaryon	9	F13w x F13-3	13	2	0

the inferred genetic similarity of the participating nuclei as occurred between different secondary mycelia.

Limited replacement of the homokaryon occurred, apparently in the absence of nuclear migration, (Fig. 5.4i) as shown by the results of genotypic analysis (Table 5.17). In these cases, the inoculated heterokaryon could be recovered from the formerly homokaryotic region only in irregular patches associated with superficial mycelial fans and superficial or submerged cords which traversed the confrontation zone.

Nuclear migration was, by contrast, only implicated in five pairings wherein almost all of the formerly homokaryotic region contained abundantly clamped secondary phase hyphae which terminated in an arc of aerial cords and tufts near the plate margin. This distribution was very similar to that observed in mating-type compatible homokaryon pairings. Strip and peripheral plug subcultures yielded a single heterokaryon which was identified as being of the inoculated type in two pairings and of a composite type (Fig. 5.4j) in the remaining three. Both of the former pairings showed an apparent diminished intensity of heterogenic incompatibility with time as the inoculated heterokaryon became established within the formerly homokaryotic region. Analysis of interaction class 3, in which sib or non-sib nuclear selection could theoretically occur, failed to reveal the presence of any composite heterokaryon.

Table 5.17. Genotypic analysis of P. velutina secondary/primary mycelial pairings.

Interaction class (see Table 5.16)	Number of interactions analysed	Number and identity of heterokaryotic genotypes detected in formerly homokaryotic regions			Number of interactions in which the inoculated homokaryon was produced by plug subculturing the periphery of the formerly homokaryotic region
		ONE			
		Inoculated type only			
		By apparent replacement	By apparent nuclear migration	Composite type only	
1	7	3	0	0	7
2	7	0	0	1	6
3	7	5	0	0	7
4	7	4	0	0	6
6	7	0	2	2	3
5+7	7	6	0	0	7

P. laevis - RESULTS

CULTURAL CHARACTERISTICS

Macroscopically, primary mycelia differed from those of P. velutina in the following ways: a single colour was common to all isolates, i.e. apricot-orange, of a uniform, mottled or concentrically zonate distribution; a greater mycelial density occurred near the inocula; the colony margin was less fimbriate and comprised narrower more closely packed hyphae (Fig. 5.5a). A ubiquitous feature, absent in all P. velutina mycelia, was the occurrence of inflated hyphal spirals containing relatively dense vacuolated protoplasm, sometimes terminating in monilioid compartments and resembling spindle cells produced during secondary mycelial interactions of C. versicolor (Rayner & Todd, 1979). Regions of straight hyphae bore occasional pseudoclamps or apparently true clamp connections (not verified under phase contrast optics), but these were never in whorls and always separated by several clampless compartments.

Field isolates bore a similar morphological relationship to primary mycelia to that observed in P. velutina, i.e. their hyphae were generally straighter, less branched, regularly bore clamp connections (very rarely in pairs) and had a higher radial extension rate.

Laboratory-produced basidiomata resembled those occurring naturally and occurred on field isolates, primary mycelia (ca. 75%) and nearly all paired combinations of the latter. Basidiospores were collected after 4 weeks dark

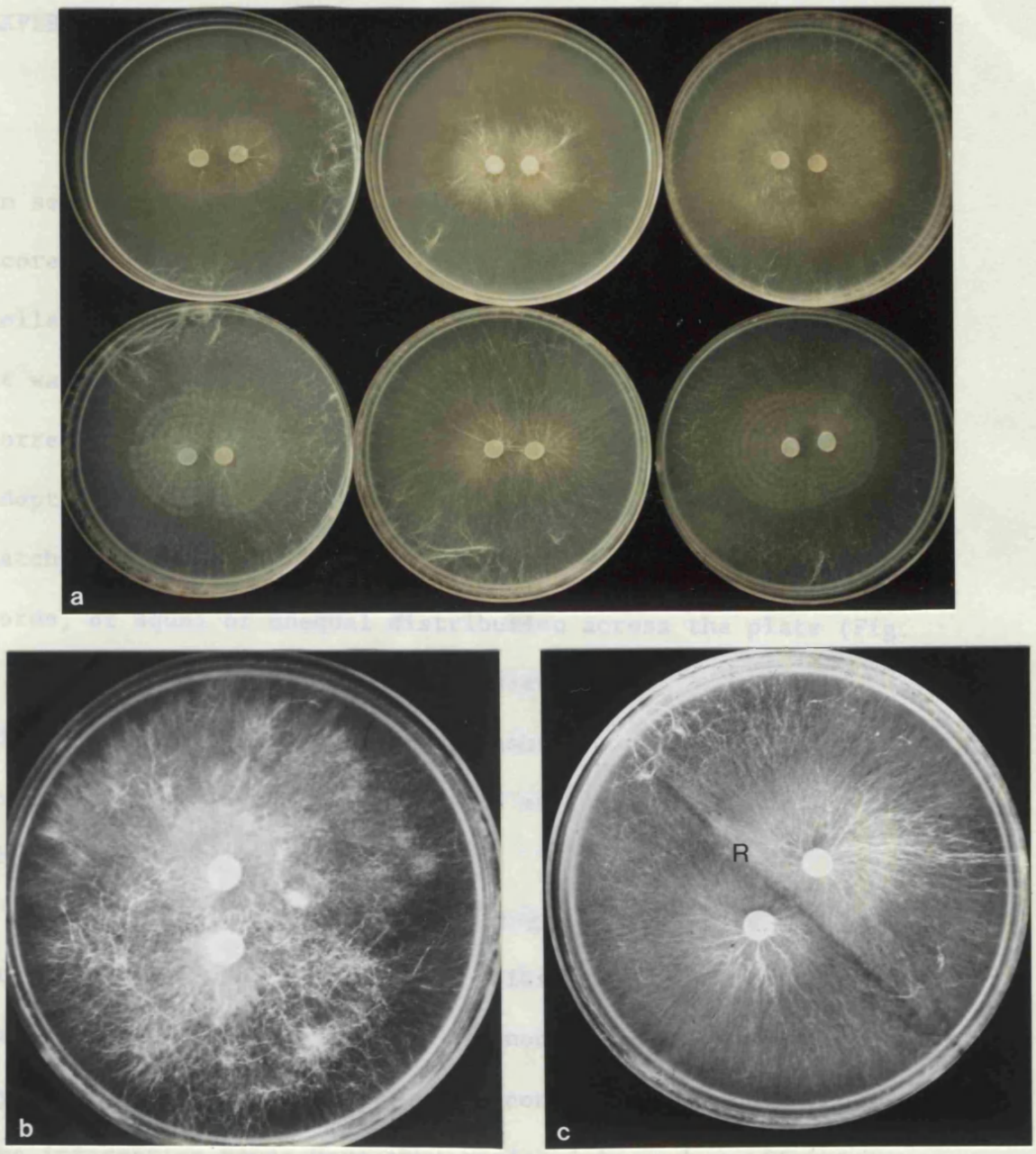


Fig. 5.5. P. laevis primary mycelial morphology and interactions. (a) Range of sib morphology. Sib mating-type (b) compatible and (c) incompatible pairings showing hyaline rejection zone (R).

incubation at 20°C followed by a similar period of ambient (15-25°C) incubation on the laboratory bench.

EXPERIMENTAL PAIRINGS BETWEEN PRIMARY MYCELIA

Mating-type compatible reactions between sibs resulting in secondary mycelial establishment were relatively easy to score. Macroscopic lysis was visible in certain pairings of one collection (M1), although unlike its counterpart in P. velutina, it was restricted to the confrontation zone as seen in certain corresponding interactions of S. rugosum. The main criterion adopted for secondary mycelial presence was the development of patchy aerial mycelium, sometimes aggregated in tufts and/or cords, of equal or unequal distribution across the plate (Fig. 5.5b). Microscopically, this was accompanied by the occurrence of regularly clamped hyphae throughout the pairing and exclusively within the aerial tufts and cords which resembled those of field isolates.

Subcultures from such pairings and weak interactions of putatively mating-type compatible sibs yielded mycelia which failed to intermingle with either inoculated primary mycelial type, indicating the presence of secondary mycelium throughout. Two interaction zones were thus produced in such confirmatory tests, each consisting of a ridge of bulked aerial mycelium.

Interactions which failed to develop the patchiness indicative of mating-type compatibility either remained morphologically distinct, although sometimes almost indistinguishably so, or produced a narrow (ca.2mm) hyaline rejection zone (Fig. 5.5c). Subcultures of these pairings

failed to produce any uninoculated mycelial types indicating mating-type incompatibility.

Both progeny sets comprised two mating-type classes (Tables 5.18, 5.19) indicating a unifactorial homogenic I.S. and non-sib pairings revealed that four different mating-type factors were present in the two collections. P. laevis is therefore diaphoromictic. The progeny set of twenty (M1) contained approximately equal numbers of the two mating-type factors, whereas that of fifteen (CW1) contained one factor that was twice as frequent as the other.

Sibs derived from laboratory-fruiting primary mycelia intermingled with each other and with their parental isolates, giving rise to interactions that were indistinguishable from control pairings.

Interactions within progeny sets derived from laboratory-fruiting secondary mycelia resembled those of the parental primary mycelia against their sibs. Two mating-type classes were revealed therein and inter-class pairings produced secondary mycelia with one parental primary mycelial type, but not the other, indicating that the two mating-type factors of the parental isolates segregated within their progeny.

EXPERIMENTAL PAIRINGS OF SECONDARY MYCELIA

Field isolates CW1w and M1w interacted aerially to produce a ridge of bulked mycelium with an underlying zone of luteous to sienna pigmentation. Field isolate/progeny primary mycelium and sib-composed secondary/sib-related primary mycelial interactions resulted in varying degrees of aerial mycelial

Table 5.18. Sib interactions of *P. laevis* M1.

	Isolate codes																		
1	10	8	16	6	5	2	11	17	12	14	3	13	15	20	4	7	9	18	19	
I	H	W	D	W	D	W	H	W	C	C	C	C	C	C	C	C	C	C	C	1
	I	D	D	D	D	H	H	H	C	C	C	C	C	C	C	C	C	C	C	10
		I	H	D	D	W	H	D	C	C	C	C	C	C	C	C	C	C	C	8
			I	H	H	H	D	D	C	C	C	C	C	C	C	C	C	C	C	16
				I	D	D	H	D	C	C	C	C	C	C	C	C	C	C	C	6
					I	W	H	H	C	C	C	C	C	C	C	C	C	C	C	5
						I	D	D	C	C	C	C	C	C	C	C	C	C	C	2
							I	D	C	C	C	C	C	C	C	C	C	C	C	11
								I	C	C	C	C	C	C	C	C	C	C	C	17
									I	W	H	D	H	D	H	H	H	D	H	12
										I	D	W	W	H	W	W	D	H	W	14
											I	W	D	D	D	H	H	H	D	3
												I	D	D	W	D	H	W	W	13
													I	D	D	W	D	W	D	15
														I	D	D	H	W	D	20
															I	W	D	H	W	4
																I	H	W	W	7
																	I	W	W	9
																		I	H	18
																			I	19

I: intermingling

C: secondary mycelial establishment

D: deadlock between morphologically distinct mycelia

H: hyaline rejection zone

W: weak interaction

Table 5.19. Sib interactions of *P. laevis* CW1.

Isolate codes															
15	10	9	8	7	6	3	2	1	11	14	13	12	5	4	
I	W	W	D	H	D	W	D	D	D	C	C	C	C	C	15
	I	D	D	H	D	D	D	W	D	C	C	C	C	C	10
		I	D	D	D	D	D	W	D	C	C	C	C	C	9
			I	D	H	D	D	W	D	C	C	C	C	C	8
				I	H	D	D	W	D	C	C	C	C	C	7
					I	D	H	H	D	C	C	C	C	C	6
						I	D	W	H	C	C	C	C	C	3
							I	W	D	C	C	C	C	C	2
								I	W	C	C	C	C	C	1
									I	C	C	C	C	C	11
										I	H	D	D	D	14
											I	D	D	H	13
												I	H	H	12
													I	W	5
														I	4

I: intermingling
C: secondary mycelial establishment
D: deadlock between morphologically distinct mycelia
H: hyaline rejection zone
W: weak interaction

bulking at the confrontation zone without accompanying pigmentation. Regularly clamped hyphae were observed within the formerly homokaryotic region indicating a possible role for the Buller Phenomenon in P. laevis.

C. puteana - RESULTS

CULTURAL CHARACTERISTICS

The germling mode of C. puteana was characterized by clampless sinuous hyphae with frequent branches. In the material studied, termination of this mode was marked by the production of aerial and superficial elements which were straight with whorled clamp connections (c.f. Kemper, 1937). The transition became macroscopically apparent at the advancing zone, because the novel hyphal type projected from an otherwise appressed, dense and even margin, thereby conferring upon it a more raised and fimbriate aspect. Microscopically, such hyphae could be traced back centipetally into the colony where their branches were of the germling type. Most progeny sets contained members which had a relatively low radial extension rate and one set (RC1) contained many such isolates, all of which produced few wide, straight clamped hyphae. More rapidly extending RC1 progeny occasionally reverted to germling mode on subculture, a switch which could be reversed by repeated subculturing.

In contrast with the other species in the present study, many primary mycelia of C. puteana had aerial components which fragmented to produce abundant arthroconidia (oidia). This

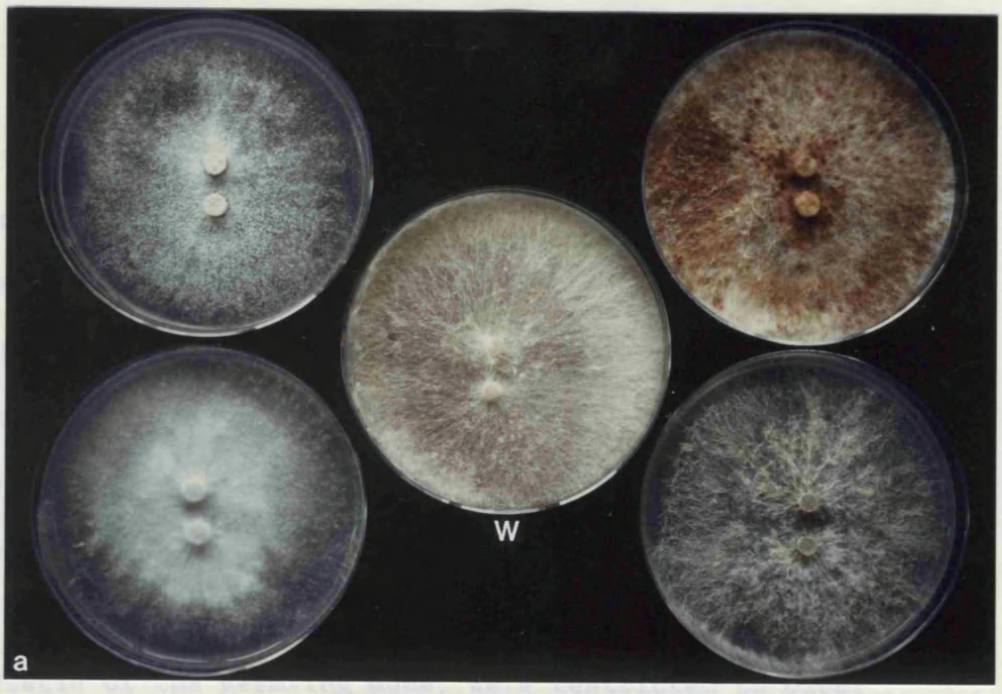
conferred a generally farinaceous to granular aerial morphology which was particularly well developed within the progeny of RC1.

Most mats were initially white then pale luteous, although some failed to change and others became irregularly blotched and speckled with luteous to chestnut pigmentation (Fig. 5.6a). Isolates with relatively sparse aerial growth often showed mycelial aggregations in the form of radiating flattened cords which were less substantial and more easily severed then those observed in cultured P. velutina.

In marked contrast with C. puteana progeny derived from woodland sites were the sibs of basidioma RR1, which was of domestic origin, having been collected from beneath wet floorboards. These showed relatively slight cultural variation (Fig. 5.8a) and initially were uniformly hyaline-white with a relatively high extension rate. Arthroconidia were not observed and the texture of the mat, the most variable visible feature, ranged from silky to plumose with a fringed or bayed margin. This consisted of relatively widely separated straight hyphae bearing whorled clamp connections. On reaching the edge of the dish, abundant aerial mycelium was produced which increased in density and dark pigmentation until it was crustose, black and exuding umber droplets. Thereupon four isolates developed tuberculate basidiomata, albeit with scanty basidiospore production.

Field isolates of woodland collections (U1, U8 and RC1) had a higher extension rate and were less dense than corresponding primary mycelia (Fig. 5.6a). They also produced arthroconidia, although to a lesser degree, and superficial radial mycelial cords which sometimes terminated in fertile

Fig. 5.6. C. puteana of woodland origin showing morphology and mycelial interactions. (a) Range of sib primary mycelial morphology and (W) parental wood isolate. (b-d) Sib mating-type compatible pairings showing (b) secondary phase hyphae (SH) emerging from one end of a primary mycelial confrontation zone (Z). Bar marker represents 1mm. This results in (c,d) secondary mycelial development both within (SMW) and around (SMA) the restricted margins of regions formerly exclusively occupied by resident primary mycelia, except when (d) pigmented rejection (R) develops between emerging secondary mycelium and a resident "blocker" primary mycelium (BPM). (e,f) Sib mating-type incompatible pairings showing (e) deadlock and (f) near-uniform morphology. (g,h) Confirmatory testing of sib pairings from a single progeny set. (g) Secondary mycelium (SM) between mating-type compatible primary mycelial precursors (A,B). (h) Presumed heterokaryon (H) subcultured from (f) between mating-type incompatible primary precursors (X,Y). (i) Pigmented and bulked rejection zone in secondary mycelial pairing. (j) Secondary/primary mycelial pairing with confrontation zone (C) overarched by secondary mycelial cords (arrowed) penetrating the primary mycelium.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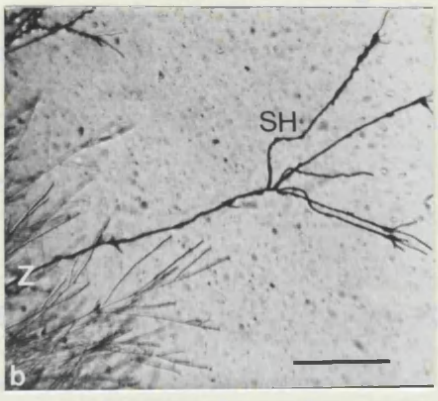
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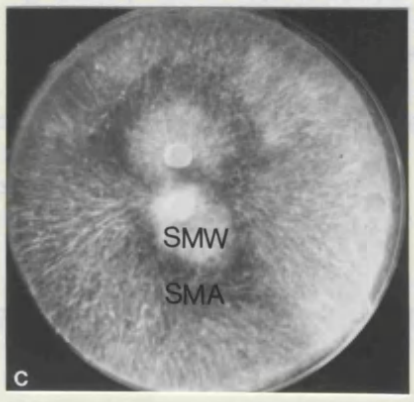
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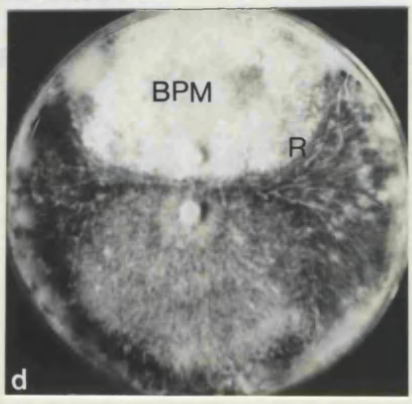
BPM
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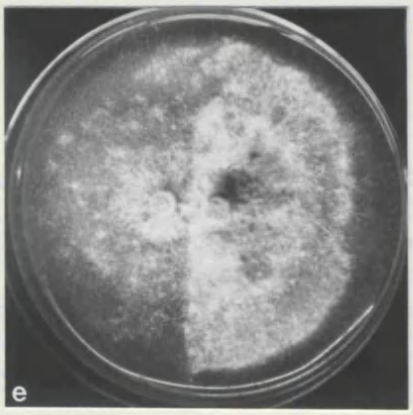
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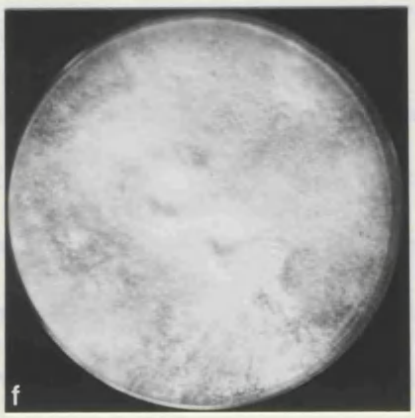
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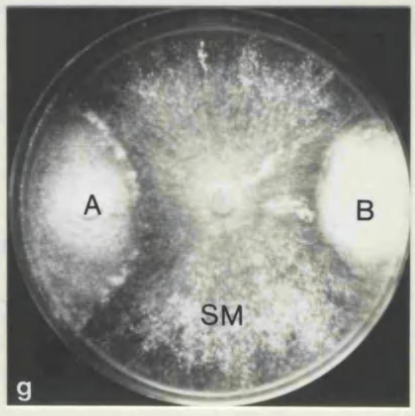
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SM
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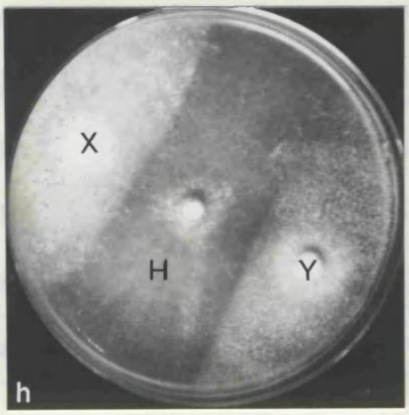
X
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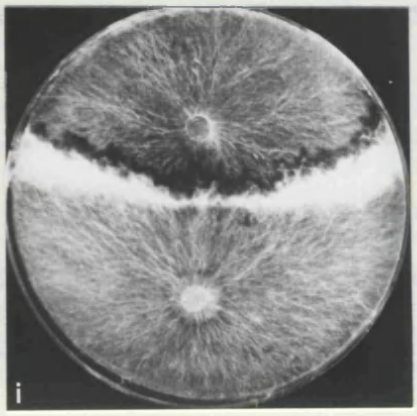
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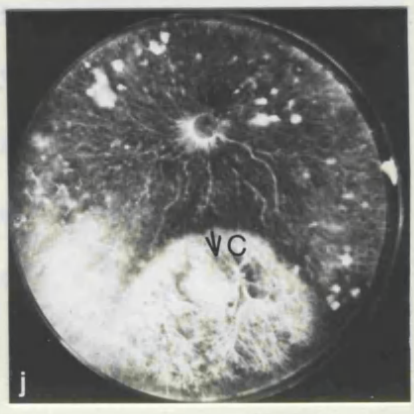

VC
j

wild-type basidiomata on the side of the dish. Initially the mat was hyaline-pale luteous, but as mycelial density increased on reaching the edge of the dish, pigmentation darkened through luteous to sienna or umber. Such a change was only observed in approximately 10% of primary mycelia of woodland origin.

Microscopically, field isolates resembled those of RR1 primary mycelia inasmuch as straight, wide, whorled clamp-bearing hyphae were more frequent than in primary mycelia of woodland origin. Hyphae of the narrow sinuous form, characteristic of the germling mode, were restricted to older more central regions of the mat. The field isolate RR1 resembled its progeny primary mycelia in all respects except that it failed to fruit in culture.

EXPERIMENTAL PAIRINGS BETWEEN PRIMARY MYCELIA DERIVED FROM WOODLAND COLLECTIONS

Lytic regions were not observed in pairings of *C. puteana* sibs. In many pairings however, a persistent narrow ($\leq$2mm) region of bulked superficial mycelium was formed centrally within the confrontation zone and was visible after 6 days incubation. Originating therein, morphologically distinct rapidly extending hyphae grew out into unoccupied medium adjacent to the ends of the confrontation zone (Fig. 5.6b). This sequence, although sometimes with indistinct macroscopic bulking, was interpreted as evidence for a mating-type compatible interaction yielding secondary mycelium with cultural characteristics of a field isolate.

Tracing the outline of interacting primary mycelia on to the underside of the Petri dishes when the emergent secondary phase was first sighted revealed that one or both residents may cease hyphal extension at about this time. This often led to an a symmetrical pattern of secondary mycelial establishment (see Fig. 5.7) that was not always explicable in terms of the difference in radial extension rates between the interacting primary mycelia. For example, the faster extending primary mycelium may be the only one of the pair that ceases extension, thereafter rendering more of the immediately adjoining unoccupied medium available for secondary phase growth than the isolate against which it is paired. Confirmatory testing of 6-day-old mating-type compatible interactions demonstrated that whilst distinct secondary mycelium was always generated from subcultured interaction zones, it may fail to be so produced from arrested colony margins. However secondary mycelium was never detected at this time by subculturing margins which subsequently continued to extend.

After a total of 8 days incubation, numerous secondary mycelial fans, apparently initiated by "point growth" were visibly projecting from those colony margins that had produced the secondary phase on subculturing at 6 days incubation. The eldest of these occurred at sites proximal to the confrontation zone, whilst successively younger fans emerged with increasing distance around the circumference of the resident mycelium. However continued incubation resulted in repeated confluence between the two eldest secondary phase regions, originating from the ends of the confrontation zone, and the younger marginal secondary fans, thus obscuring the form of the latter. In the

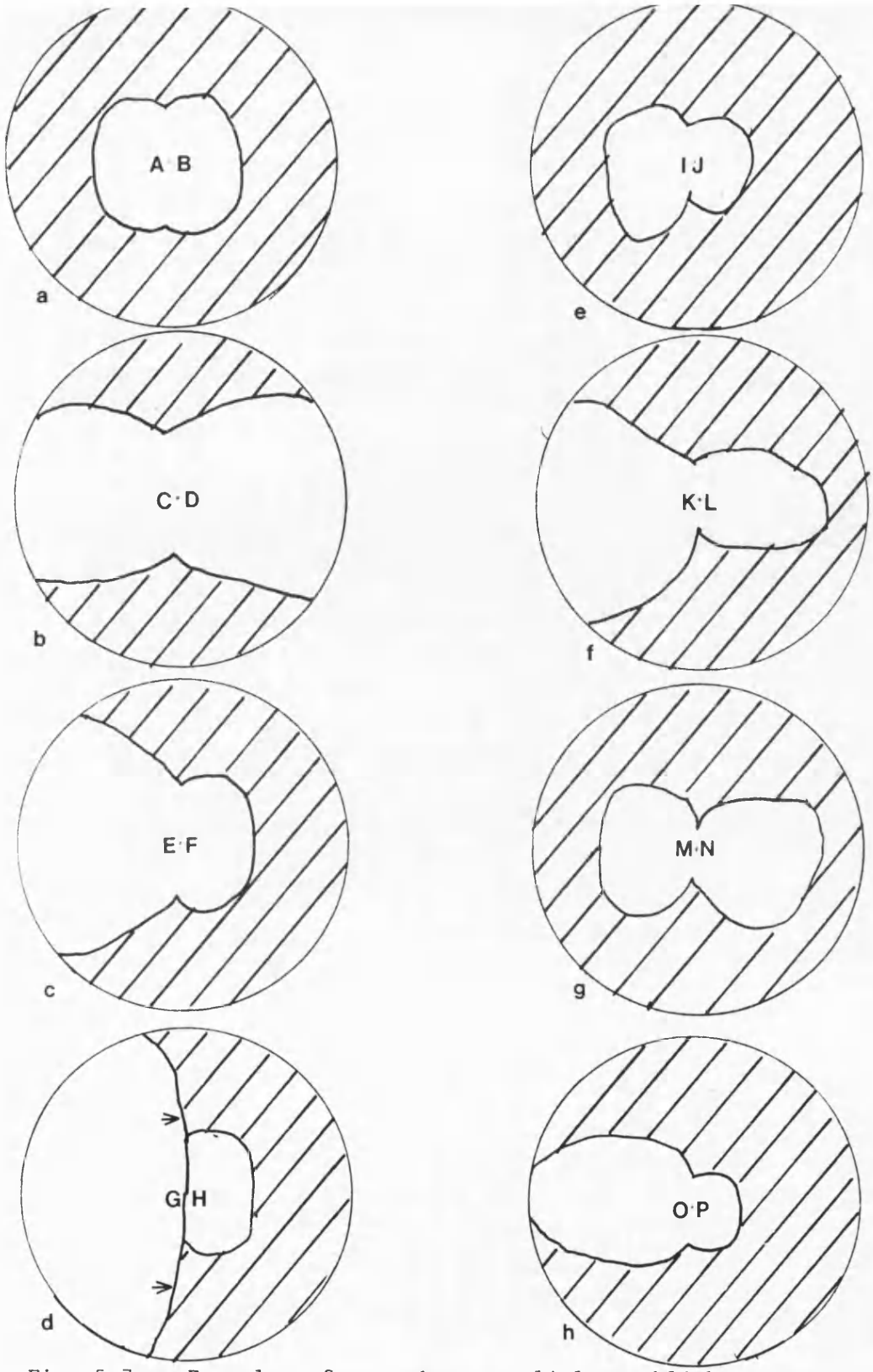


Fig. 5.7. Examples of secondary mycelial establishment patterns between paired C. puteana primary mycelia of woodland origin. Regions exclusively occupied by secondary mycelium (hatched) are shown in mating-type compatible pairings (a-d) between mycelia with ca. equal radial extension rates and (e-h) such that mycelia inoculated at I,K,M,O have the higher radial extension rates. After ca. 6 days incubation, mycelia inoculated at A,B,F,H,I,J,M,P ceased radial extension. (d) Pigmented rejection (arrowed) at the interface of secondary mycelium and precursive "blocker" primary mycelium.

absence of such repeated marginal fan production, secondary mycelial fronts extended from the confrontation zone margins as far as possible within unoccupied medium. Their final extent seemed to be dependent on the combination of isolates present. At one extreme one or both primary mycelia were encircled (Fig. 5.6c), as reported in Serpula by Harmsen (1960), whilst at the other, the secondary phase barely established itself between the residents within and at the ends of the confrontation zone (Fig. 5.7). Some mating-type compatible combinations of U8 sibs resulted in one showing "blocker" behaviour. This was indicated by continued radial extension of one primary mycelium throughout an interaction and the development of a narrow ($\leq$3mm) rust to chestnut pigmented zone which demarcated it from the other mycelial phases in the Petri dish (Figs. 5.6d, 5.7d).

Confirmatory testing of 5-week-old interactions was carried out by subculturing regions of distinct secondary mycelium and regions lying within the primary residents. In almost all cases, the subcultures produced distinct, sparse rapidly extending mycelium, interpreted as the secondary phase, which intermingled when derived from a common interaction. Confrontation zones between secondary mycelium and stock isolates in confirmatory tests were either pigmented rust to chestnut, or more commonly merely associated with an increased mycelial density within the formerly homokaryotic region (Fig. 5.6g).

Regions of interaction plates that were occupied by secondary mycelium showed an increasing intensity of pigmentation when placed on the laboratory bench in common with woodland field isolate mycelia. Their final sienna-umber colour

facilitated rapid location of mating-type compatible pairings within a stack of Petri dishes containing interacting C. puteana primary mycelia.

Sib pairings which failed to initiate recognizable outgrowth of secondary mycelium from the confrontation zone rarely produced a discrete interaction zone. Indeed, only two examples of such a zone, both within progeny of U8, were recorded, i.e. one hyaline and one of sienna pigmentation. The more frequently encountered interaction types were deadlock, in which the isolates maintained their morphological differences with or without a bulked interface (Fig. 5.6e) and weak, in which the isolates were originally morphologically very similar.

Three types of U8 and RC1 pairings of putatively mating-type incompatible sibs were exceptional. One was detected after 4 or 5 weeks incubation and showed an annular marginal region of altered morphology resembling that of a mating-type compatible interaction; the second and third, also seen in U1, were only revealed after 9-13 weeks incubation and involved a gradual increase in aerial mycelium, either spreading throughout one isolate from the confrontation zone, or across the entire plate, both of which resulted in an almost uniform subfelty or felty aerial aspect (Fig. 5.6f). All three types of interaction thereby acquired the morphology of one or neither of the originally paired isolates. Twenty five subcultures from such interactions yielded a range of mycelial morphology which in confirmatory tests against stock primary mycelia produced a range of responses. At one extreme, an intermingling response indicated replacement of one resident primary mycelium by the other, whilst at the other extreme, pigmented rejection

responses coupled with a relatively high radial extension rate and sparse aspect satisfied the criteria for secondary (or at least heterokaryotic) mycelial establishment, albeit from isolates of putatively identical mating-type (Fig. 5.6h). Between these extremes were mycelial types whose constitution was difficult to interpret because they were of similar, but not identical, morphology to one or both stock primary mycelia and interacted weakly against them. A few subcultures yielded apparently heterokaryotic mycelium which then reverted to one or other primary type before encountering the stock isolates on confirmatory test plates.

Despite detection of a mycelial type satisfying the confirmatory test of a secondary mycelium in six pairings of putatively mating-type incompatible isolates, each progeny set was assigned to a pair of mating-type classes in accordance with a unifactorial homogenic I.S. (Tables 5.20-5.22).

All non-sib pairings produced secondary mycelium in the manner of mating-type compatible sibs indicating multiple mating-type specifities and hence a diaphoromictic breeding system.

EXPERIMENTAL PAIRINGS OF SECONDARY MYCELIA DERIVED FROM WOODLAND COLLECTIONS

The pigmentation intensity of interaction zones was greatest between paired field isolates which always produced an intervening umber-chestnut region. Corresponding zones between laboratory synthesized sib-composed sib-related secondary mycelia and between sib-composed and parental field isolates

Table 5.20. Sib interactions of *C. puteana* U1.

	Isolate codes														
	1	3	5	7	8	14	13	2	4	6	9	10	11	12	15
1	I	W	M	D	M	W	M	C	C	C	C	C	C	C	C
3		I	W	D	W	D	W	C	C	C	C	C	C	C	C
5			I	D	W	U	D	C	C	C	C	C	C	C	C
7				I	D	D	D	C	C	C	C	C	C	C	C
8					I	D	D	C	C	C	C	C	C	C	C
14						I	D	C	C	C	C	C	C	C	C
13							I	C	C	C	C	C	C	C	C
2								I	U	U	M	U	M	W	U
4									I	U	W	M	U	D	W
6										I	M	M	U	D	W
9											I	W	W	U	U
10												I	U	M	W
11													I	U	U
12														I	M
15															I

I: intermingling

C: secondary mycelial establishment initially from the ends of the confrontation zone

D: deadlock between morphologically distinct mycelia

U: D giving rise to uniform morphology over the plate after 9-13 weeks incubation

M: mound of bulked aerial mycelium

W: weak interaction

Table 5.21. Sib interactions of C. puteana U8.

	Isolate codes																		
9	7	19	20	3	8	6	12	13	17	4	14	11	2	5	15	10	16	18	1	
I	U	U	U	U	U	D	D	D	U	U	A	U	C	C	C	C	C	C	C	9
	I	U	U	U	U	U	U	U	U	U	U	U	C	C	C	C	C	C	C	7
		I	U	U	A	D	U	U	U	U	P	U	C	C	C	C	C	C	C	19
			I	D	A	D	D	A	D	U	U	D	C	C	C	C	C	C	C	20
				I	U	A	U	U	U	U	D	D	C	C	C	C	C	C	C	3
					I	U	U	U	U	U	A	D	C	C	C	C	C	C	C	8
						I	D	U	U	D	A	D	C	C	C	C	C	C	C	6
							I	D	U	U	D	A	C	C	C	C	C	C	C	12
								I	U	D	A	D	C	C	C	C	C	C	C	13
									I	U	U	U	C	C	C	C	C	C	C	17
										I	D	U	C	C	C	C	C	C	C	4
											I	U	C	C	C	C	C	C	C	14
												I	C	C	C	C	C	C	C	11
													I	U	U	U	U	U	D	2
														I	U	U	U	U	U	5
															I	U	A	A	U	15
																I	U	U	H	10
																	I	U	D	16
																		I	U	18
																			I	1

I: intermingling

C: secondary mycelial establishment initially from the ends of the confrontation zone

D: deadlock between morphologically distinct mycelia

A: D giving rise to annular marginal region of altered morphology after ca. 4 weeks incubation

U: D giving rise to uniform morphology over the plate after 9-13 weeks incubation

H: hyaline rejection zone

P: pigmented rejection zone

M: mound of bulked aerial mycelium

W: weak interaction

Table 5.22. Sib interactions of *C. puteana* RC1.

	Isolate codes														
	12	14	13	7	3	1	2	5	15	8	6	10	11	9	4	
12	I	W	W	U	W	D	C	C	C	C	C	C	C	C	C	12
14		I	W	U	U	D	C	C	C	C	C	C	C	C	C	14
13			I	D	U	D	C	C	C	C	C	C	C	C	C	13
7				I	D	D	C	C	C	C	C	C	C	C	C	7
3					I	D	C	C	C	C	C	C	C	C	C	3
1						I	C	C	C	C	C	C	C	C	C	1
2							I	D	U	D	U	D	D	U	A	2
5								I	U	D	W	U	U	D	U	5
15									I	W	D	W	U	U	U	15
8										I	W	A	W	W	W	8
6											I	W	W	W	U	6
10												I	W	U	U	10
11													I	W	W	11
9														I	D	9
4															I	4

I: intermingling

C: secondary mycelial establishment initially from the ends of the confrontation zone

D: deadlock between morphologically distinct mycelia

A: D giving rise to annular marginal region of altered morphology after *ca*. 4 weeks incubation

U: D giving rise to uniform morphology over the plate after 9-13 weeks incubation

W: weak interaction

were paler (fulvous to rust) especially where the mycelia had a common nuclear type. These colours were also sometimes partially or totally obscured by a bulked ridge of aerial mycelium (Fig. 5.6i). All expressions of heterogenic incompatibility between secondary mycelia were visibly more striking than any of those displayed by mating-type incompatible primary mycelia.

Interactions between secondary and primary mycelia derived from a single progeny set, or between field isolates and progeny primary mycelia, involved a mound of bulked aerial mycelium at the interaction zone, rarely with underlying fulvous to rust pigmentation. In nearly every case the secondary mycelium could be seen overarching the interaction zone and encroaching into the primary mycelium beyond, often in the form of aerial cords (Fig. 5.6j). Although nuclear migration cannot be discounted from such interactions, these observations suggest a significant role for intrusive growth in bypassing the interaction zone.

EXPERIMENTAL PAIRINGS OF PRIMARY MYCELIA DERIVED FROM A DOMESTIC COLLECTION

Mating-type compatible interactions of RR1 progeny were relatively difficult to distinguish. This was due in part to their lack of cultural variation, and also to the relatively small morphological change accompanying putative secondary mycelium development. Consequently criteria for mating-type compatibility were not so readily established as with woodland collections. Secondary mycelium was tentatively judged to be

present if a diminution of morphological differences between the paired isolates was accompanied by a slight superficial bulking of the confronation zone (Fig. 5.8b). An additional criterion was provided by superficial mycelial cord formation in various orientations which obscured the preceding radial aspect of superficial silky elements which persisted in control pairings.

Confirmatory tests proved relatively unhelpful, again due to the morphological similarities and lack of visible interaction between members of the progeny set and putative secondary mycelia. Many of these interactions could only be described as weak, but the sibs could nevertheless be assigned to a pair of mating-type classes (Table 5.23) albeit on relatively slender morphological criteria. Interactions of sibs ascribed to a common mating-type class never resulted in a discrete zone of demarcation, but slight morphological differences, characteristic of the paired isolates, were usually retained on either side of the confrontation zone (Fig. 5.8c). However, ten pairings were exceptional in this respect, resembling mating-type compatible interactions although not conforming to any obvious pattern. One of these involved a fan-shaped outgrowth from the ends of the confrontation zone whilst the remaining colony margins were arrested. Confirmatory testing could provide no new information about the identity of such mycelial types.

Germination of progeny derived from fruiting RR1 primary mycelia was accelerated by a small mycelial inoculum of RC1-6. The resulting isolates intermingled completely when paired against their sibs, their parental mycelial types and themselves in control interactions.

Table 5.23. Sib interactions of *C. puteana* RR1.

	Isolate codes													
2	14	1	5	7	3	6	9	11	15	10	8	12	13	4	
I	D	C	C	D	D	C	C	C	C	C	C	C	C	C	2
	I	D	D	D	D	C	C	C	C	C	C	C	C	C	14
		I	C	D	W	C	C	C	C	C	C	C	C	C	1
			I	D	C	C	C	C	C	C	C	C	C	C	5
				I	W	C	C	C	C	C	C	C	C	C	7
					I	C	C	C	C	C	C	C	C	C	3
						I	D	D	D	C	W	D	D	C	6
							I	W	D	D	W	W	W	W	9
								I	D	D	W	C	D	W	11
									I	D	W	W	W	D	15
										I	D	C	D	D	10
											I	C	W	W	8
												I	D	D	12
													I	D	13
														I	4

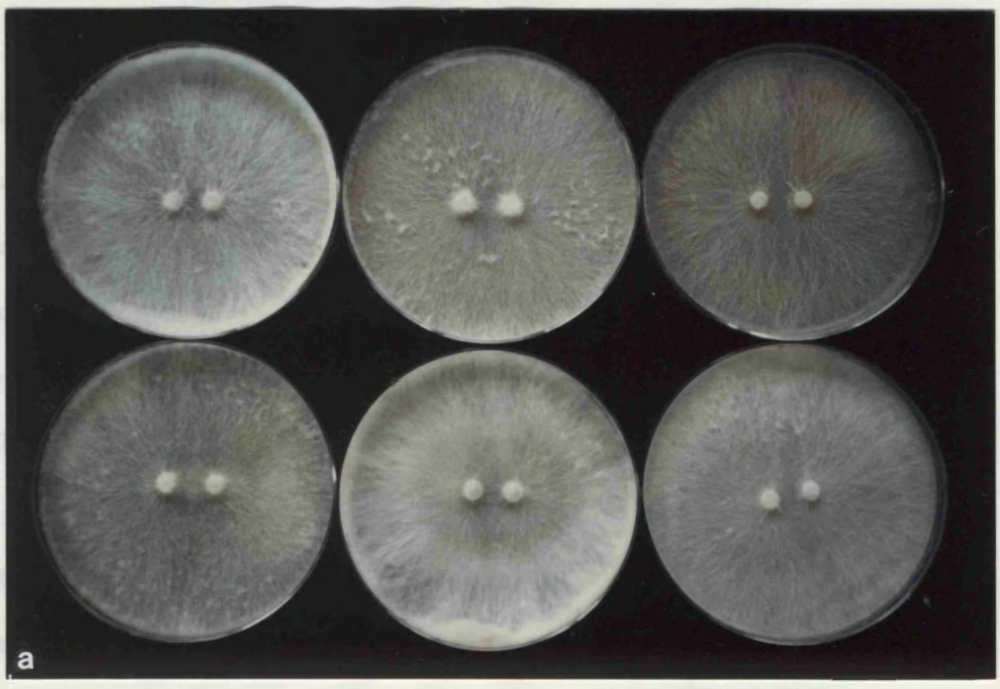
I: intermingling

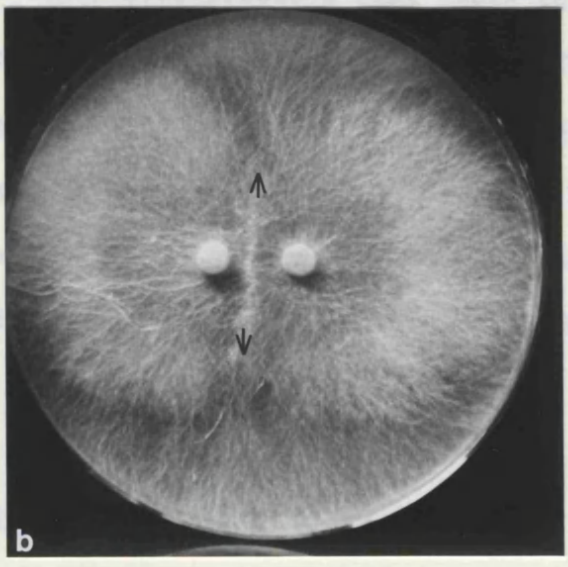
C: secondary mycelial establishment or heterokaryon production initially from the ends of the confrontation zone

D: deadlock between morphologically distinct mycelia

W: weak interaction

Fig. 5.8. C. puteana of domestic origin showing primary mycelial morphology and interactions. (a) Range of sib morphology. (b) Sib mating-type compatible pairing showing bulked confrontation zone produced between the resident primary mycelia preceding fan-shaped secondary mycelial development from its ends (arrowed). (c) Mating-type incompatible sib pairing. (d,e) One dish viewed from (d) below and (e) above showing pigmentation (P) underlying a mycelium of domestic origin paired against one of woodland origin.


a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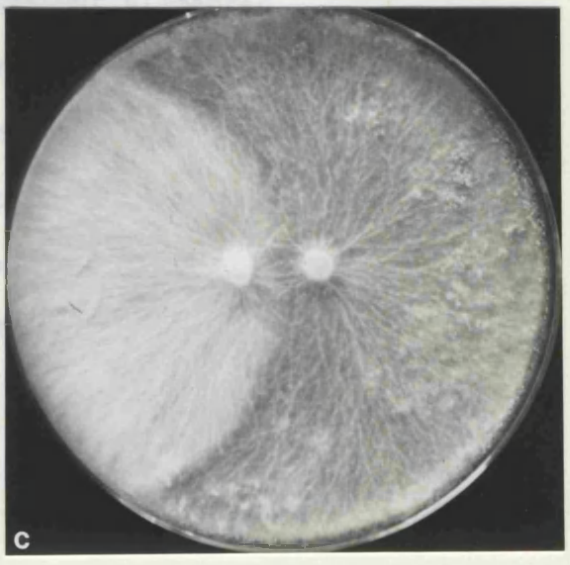
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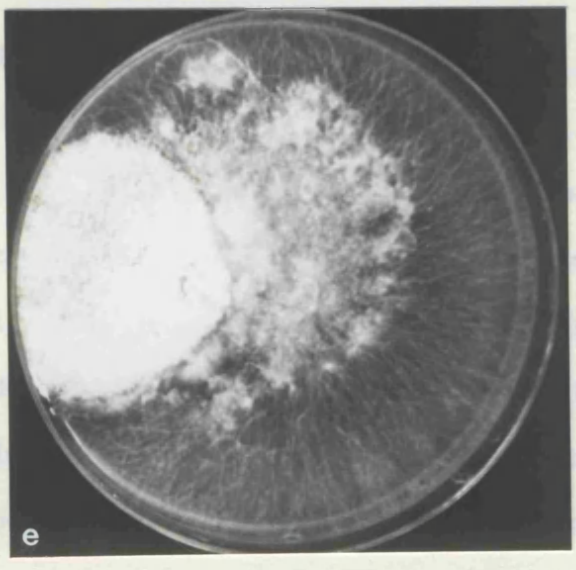
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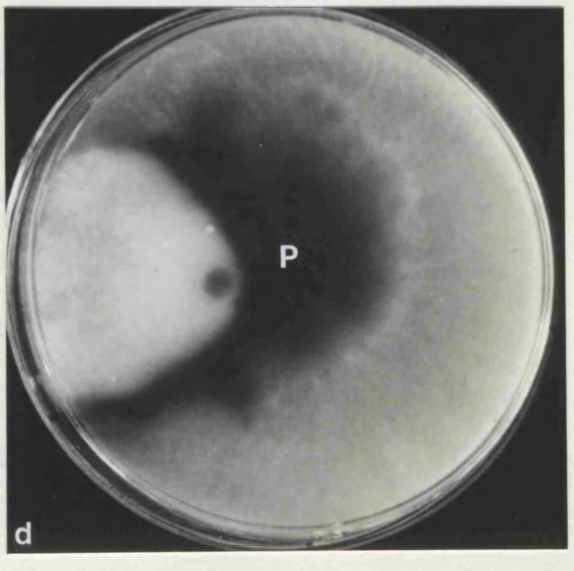

P
d

Interactions between RR1 isolates and those of woodland origin always resulted in a sienna to chestnut interaction zone, widest near its central point, which expanded unilaterally or bilaterally. The most frequent outcome was a unilateral expansion into the domestic isolate (Fig. 5.8d,e), which on subculture, however, still produced the inoculated domestic mycelial type. This failed to occur in two interactions of U1-3 and one of U8-7 in which a bilaterial expansion of the pigmented region took place. Although subcultures from the RR1 side of the interactions still generated RR1 mycelial types, those from the other side produced flat sepia to chestnut pigmented mycelium with a dense even margin. This failed to reach the edge of the dish and produced concolorous pigmented interaction zones within the adjacent margins of both putatively parental isolates. A third type of interaction occurred between RR1-13 and RC1-1 in which the former overarched and replaced the latter as revealed by confirmatory testing.

DISCUSSION

POSSIBLE CONSEQUENCES OF HOMOKARYOTIC FRUITING IN THE FIELD

There are many similarities between fruiting primary mycelia of outcrossing and non-outcrossing forms, but the ability of the former to undergo heterokaryosis potentially endows them with a unique property. In the unlikely event of being produced in the field, their basidiomata may not only function in basidiospore production and dispersal, but also in entrapment of conspecific non-sib-related basidiospores, thereby

fulfilling the role of a fungal flower. The arrival of several mating-type compatible basidiospores may therefore convert a homokaryotic basidioma into a mosaic of small genetically and physiologically distinct heterokaryons, each producing meiotically-derived basidiospores. This in turn could lead to greater numbers of mating-types being present in the basidiospores of a single basidioma than in the contiguous mycelium. Since the basidioma would be present before heterokaryosis, this would circumvent Buller's (1931) argument that physiological unity was necessary between many small genetically distinct conspecific units for basidiomatal morphogenesis to occur.

INTERACTIONS BETWEEN PRIMARY MYCELIA

The results showed that S. rugosum, P. velutina, P. laevis and C. puteana regulate a mode change from the primary to the secondary phase by a single breeding system, that of unifactorial diaphoromixis. However, the pattern of secondary mycelial establishment was far from similar, and indeed could serve as a taxonomic criterion for distinguishing between primary mycelia of the four species in question. The working hypothesis outlined in Chapter 1 will be invoked to try to explain these differences.

A macroscopically visible interaction is believed to be the gross result of repeated anastomosis between hyphae of different mycelia and the subsequent varying fates of the fusion compartments thus formed. Three basic series of events were detected within fusion compartments of P. velutina (see Chapter

8), i.e nuclear division/septation, septal erosion/nuclear migration and hyphal rejection. Assuming these are generally applicable to all four species under consideration, it is suggested that during mycelial intermingling most fusion compartments undergo nuclear division/septation cycles, whereas a visible expression of heterogenic incompatibility is indicative of hyphal rejection resulting in protoplasmic lysis. It is also suggested that although inhibition/appression reactions and secondary mycelial establishment are both initiated by septal erosion in, and nuclear migration from, fusion compartments, the duration of migration may vary and it may even be superseded by rejection. Migration may also be spatially restricted within a resident mycelium by regulation of septal erosion, hyphal branching and fusion frequency of recently formed heterokaryotic hyphae.

S. rugosum

In contrast with mating-type compatible pairings in S. hirsutum and S. gausapatum, those in S. rugosum were relatively slow to produce any visible indication of secondary mycelium. Paradoxically, the scattered white tufts of aerial mycelium which indicated mating-type compatibility were often preceded by lysis in the confrontation zone. Rayner & Turton (1982) reported that these zones were relatively strong and persistent within a progeny set in which the associated aerial tuft formation was relatively poor. The same set of interactions also developed numerous small appressed patches of mycelium.

In the present study, lytic zones associated with mating-type compatibility were often persistent and accompanied

by poor subsequent aerial tuft development in non-sib pairings. Therefore, in addition to their function in overriding heterogenic incompatibility, dissimilar mating-type factors may also hasten or intensify its visible expression. Such an intimate involvement of mating-type factors in the acceptance/rejection balance could explain the development of lytic zones in mating-type compatible sib pairings before those in mating-type incompatible ones. It is also consistent with non-sib combinations producing more intense regions of lysis and fewer aerial tufts than sib combinations of mating-type compatible isolates. Such visible phenomena may be the result of frequent hyphal rejection, either directly after anastomosis or following a brief period of nuclear migration. Longer periods of nuclear migration, although rare in themselves, may not always be subsequently stabilized leading to mottled regions of mycelium and a relatively slow establishment of the secondary phase within discrete tufts.

A combination of heterokaryotic aerial tufts and "small knots of hyphae" has also been observed in mating-type compatible interactions in T. cucumeris (Anderson, 1984). However, secondary mycelial establishment in this species differs from that in S. rugosum in that both morphological features are restricted to the confrontation zone suggesting that stabilized access migration occurs over relatively short distances. It seems unlikely that the secondary mycelium is able to coexist with primary mycelia in the medium and the relative speed of its establishment and radial extension seem insufficient to produce bow-tie shaped zones from the ends of

the confrontation zone as occurs in Serpula spp. (Harmsen, 1960) and C. puteana.

P. laevis

In P. laevis, the transition from primary to secondary phase was accompanied by an increased propensity for mycelial aggregation, manifested as cords and aerial tufts, but without any visible indications of access migration. Although comparisons were not made between estimates of nuclear migration rate in different mycelia, the uniform pattern of secondary mycelial development resembled that of S. hirsutum (Coates et. al., 1981, 1985b) and so may provide another example of acceptor migration. A lytic confrontation zone between mating-type compatible sibs occurred within on progeny set resembling that of S. rugosum and may similarly result from frequent hyphal rejection, either directly after anastomosis, or after a short intervening period of override, septal erosion and nuclear migration.

P. velutina

The speculated dual role of dissimilar mating-type factors in S. rugosum, both in activating and overriding heterogenic incompatibility, is equally applicable in P. velutina. However, their action in the latter did not affect the timing of its expression, but resulted in a lenticular region of intense lysis within the resident primary mycelia. This contrasted with the relatively narrow interaction zone and low intensity of rejection between most mating-type incompatible sibs. It seems likely that the extent of lysis was largely determined by override events initiating temporary nuclear migration before rejection occurred in the migration hyphae thus

formed. Secondary phase hyphal remnants were also present in the lytic region which probably resulted from destabilization of the secondary phase.

Evidence of access migration was only present within certain mating-type compatible interactions of U5 progeny. The generation of secondary mycelium from subcultures of these regions supported the suggestion that unstabilized access migration results from a partially operative morphogenetic sequence analogous to that operated by dissimilar B factors in the bifactorial diaphoromictic system.

Inbreeding, which was expected to increase the genetic similarity of sibs, resulted in less lysis and apparently greater density of secondary mycelium in mating-type compatible pairings. This is in accord with the view that lessened heterogenic incompatibility is permanently and more frequently overridden following anastomosis. Hence the balance between post-fusion rejection and secondary phase establishment was apparently shifted in favour of the latter.

C. puteana

Paired C. puteana primary mycelia showed a relatively weak expression of heterogenic incompatibility. Stabilization of the much sparser rapidly-extending secondary phase occurred relatively rapidly in this species and was not accompanied by any macroscopically visible lysis or other evidence of long distance nuclear migration. The only possible exception to this was the primary colony margin arrest preceding encirclement by the secondary phase. However in the absence of morphological changes or hyphal tip analysis it was not possible to distinguish the role of migration from that of resource

reallocation towards secondary mycelium developing from the confrontation zone. Initiation of discrete secondary phase fans from the margins of such primary mycelial pairings suggests that long distance nuclear migration, if operative, may only occur in a very small proportion of hyphae. Moreover, it was suggested that mycelial penetration or overgrowth as cords may play a larger role in establishing the secondary phase within precursive primary mycelia.

The status of apparently heterokaryotic mycelia produced by long-term incubation of some putatively mating-type incompatible pairings of C. puteana (woodland collections) is unclear as are the roles of nuclear migration and intrusive hyphal growth. The lack of heterogenic incompatibility between pairs of such isolates may allow extensive hyphal interdigitation and the nuclear division/septation response may often follow anastomosis between them. Whilst subcultures from the interaction product were sometimes clearly morphologically distinct from either precursor, they rarely showed all those features indicative of a transition to the stable secondary mode, e.g. radial extension rate, hyphal density and fruiting ability. Hyphal tip analysis may elucidate the nuclear status of such mycelia. If precursive primary types were produced from a mycelium regenerated by a single apical compartment directly by spontaneous sectoring, after the equivalent of dedikaryosis, or their genotypes were inferred from the Buller Phenomenon, then it could be concluded that the original tip was heterokaryotic. Thus there is an intriguing possibility that C. puteana may have an auxiliary, slow, perhaps acceptor type of

nuclear migration which rarely reaches full stabilization as secondary mycelium.

The morphological similarity shown within and between the primary and secondary phases of the domestic collection RR1 suggests that relatively little meiotic variation was generated in the parental basidioma. Hence it may have originated from genetically similar, perhaps sib-related, primary mycelia. This may be a frequent outcome of fruiting in confined spaces or following invertebrate-assisted basidiospore dispersal.

Although not occurring in bow-tie shaped regions, unstablilized access migration was implicated in non-sib pairings of RR1 against woodland isolates in which a pigmented zone invaded one or both isolates from the confrontation zone. The few subcultures from this zone that failed to intermingle with one or other of the interacting isolates were dense, intensely pigmented umber-chestnut, relatively slowly extending and unlike C. puteana secondary mycelia from all other sources. The existence of such a barrier hindering the establishment of stable secondary mycelium from RR1 non-sib pairings was interesting in view of the attendant morphological differences between paired isolates and the detection of homokaryotic fruiting within the domestic progeny only. This suggested that the collection RR1 may have been incorrectly identified and interfertility testing against bona fide Coniophora arida and related species may prove instructive.

If, however, RR1 proves to be interfertile with any future collection of C. puteana, then the delayed rejection response may indicate incipient speciation. However the significance of override breakdown in such a process is far from

clear. It may be the event which causes sympatric genetic isolation and hence permits independent evolution, possibly leading to a taxonomically recognizable discontinuity in morphological variation. Alternatively, an ancestral gamodeme may become partitioned by ecological specialization into more or less independently evolving gamodemes. A consequence of their accumulated genetic differences could then be an increasingly hindered override mechanism.

Unilateral nuclear migration with accompanying brownish pigmentation has also been reported within Typhula idahoensis homokaryons when paired against Typhula ishikariensis homokaryons or heterokaryons (Bruehl, Machtmes & Kiyomoto, 1975; Christen & Bruehl, 1979; Bruehl, Jacobs & Machtmes, 1983). The products of these so-called spurious matings were similar to those of RR1 non-sib interactions and indicated a role for access migration. Their radial extension rates were usually lower than those of component primary mycelia; clamp connections, which when abundant were used as a criterion for heterokaryosis, were often sparsely distributed; aerial hyphae apparently collapsed, as occurs in the bow-tie reaction in S. hirsutum, and they produced few basidiomata in culture. Some hybrids were as virulent as field isolates of either species and produced sclerotia on diseased leaves, indicating the likelihood of their natural occurrence. However, it was concluded that the two morphologically intergrading species were closely related, but interfertility tests were interpreted as producing negative results, thus supporting their continued taxonomic separation.

INTERACTIONS OF SECONDARY MYCELIA

The results indicate that collected basidiomata of S. rugosum, P. laevis, P. velutina and C. puteana were produced by secondary mycelia which were mutually heterogenically incompatible when conspecific pairings were made in culture. Subcultures taken from pairings involving one or two secondary mycelia only produced mycelial types differing from the originals when they were removed from those sides of the confrontation zone that had been inoculated with a primary mycelium. This is in accord with the hypothesis that once a secondary mycelium has been stabilized, future post-fusion responses of the mycelium to donated nuclei are restricted to the rejection or nuclear division/septation sequences.

The absence of septal erosion and hence nuclear migration within secondary mycelia of S. commune has been associated with an unspecified change in the septal wall following stabilization (Snider, 1965). Janszen & Wessels (1970) reported that septal erosion occurred within isolated hyphal wall fragments of S. commune primary mycelia when treated with R-glucanase and chitinase, but the hyphal walls retained their structural integrity. Increased R-glucanase activity has also been detected within mycelia undergoing septal dissolution, but isolated secondary mycelial wall fragments contained septa that were relatively resistant to R-glucanase and chitinase (Wessels, 1978). Moreover, when secondary mycelium exhausted glucose in the medium, R-glucanase activity rose sharply but had little effect on septal structure (Wessels & Niederpruem, 1967). Chemical analyses failed to reveal any differences between the

amounts of R-glucan and chitin in primary and secondary phase hyphal walls, which led Wessels (1978) to speculate that the phases differ with respect to the structure of their septal R-glucan. Therefore, if high R-glucanase activity can be demonstrated in the holocoenocytic heterokaryon, a similar change in septal properties will also need investigation within Stereum, Phanerochaete and Coniophora.

The role of mating-type factors in the fusion compartments of paired secondary mycelia is unclear although their override function seems to be inoperative. Nevertheless, in view of the intense, narrow lytic interaction zones of P. velutina in particular, dissimilar mating-type factors may enhance the expression of heterogenic incompatibility loci as has been suggested to be the case with interactions between primary mycelia.

From the foregoing discussion, secondary/primary mycelial interactions can be explained in terms of two components. One is the constant rejection or non-acceptance of donated nuclei by the secondary mycelium and the other is the full range of responses that the primary mycelium may make to reciprocal nuclear transfer. The former response intensity is expected to vary inversely with genetic similarity, as has been recorded between laboratory-synthesized secondary mycelia. The latter responses differ from those of two mated primary mycelia in that more than one secondary mycelium may become established within a formerly primary mycelial region or, alternatively, the pre-fusion heterokaryotic association may prevent composite combinations of genotypes becoming established as secondary mycelia.

In P. velutina and S. rugosum, the changes that occurred on the primary mycelial side of the confrontation zone were strikingly different as follows. S. rugosum was characterized in this respect in all but one experiment by heterokaryosis, often preceded by a persistent band of appressed morphology, leading to the establishment of a maximum of three secondary mycelial types within the formerly primary mycelial region.

Comparable work on secondary/primary mycelial interactions of unifactorial holocoenocytic forms is limited to studies on S. hirsutum in which heterokaryosis always occurred, but resulted in a maximum of two secondary mycelia in the formerly primary mycelial region (Coates et al., 1981, 1985; Coates & Rayner, 1985c). This was frequently preceded by a transient zone of appressed morphology, described by Coates & Rayner (1985c) as a lytic region, in which track formation could occur. The latter phenomenon seems to be replaced in S. rugosum by a more diffuse and blotchy distribution of pigment. A feature shared by both these species was the rarity of two composite heterokaryons being recovered from an interaction, although the mechanism of exclusion remains obscure. Selective exclusion of sib-composed compared to non-sib-composed heterokaryons predominated in class 3 pairings of both S. hirsutum and S. rugosum (Table 5.6). This implies that in the presence of dissimilar mating-type factors, increasing the level of genetic dissimilarity beyond that existing between sibs shifts the balance between expression and override of heterogenic incompatibility in favour of override. Indeed, direct evidence for this has been supplied in S. hirsutum isolates by Coates et al. (1985).

Clearly the foregoing does not apply to P. velutina, in which dissimilar mating-type factors seem to have a greater role in rejection as genetic dissimilarity increases from that existing between inbred sibs, to that between British non-sibs. When operative in secondary/primary mycelial interactions, their effect on rejection seems to preclude their override function in almost all encounters. Moreover, the presence of a pigmented zone of heterogenic incompatibility between mycelial cords of in situ heterokaryons and their component homokaryons reinforces the view that rejection as expressed by a heterokaryon is not merely the additive effects of that expressed by its component homokaryons (see Adams et al., 1981). In such encounters, one genotype is common to both heterokaryon and homokaryon and if the strength of heterogenic incompatibility expressed between the two was merely an additive effect, then a similarly pigmented zone would be expected between many mating-type incompatible sib combinations. That this very rarely occurred, suggests that the potential intensity of heterogenic incompatibility expression is increased when two dissimilar genotypes effect a mode change by their association in a common cytoplasm. In P. velutina, this difference seems to be great enough to reduce the chances of mating via the Buller Phenomenon to a very low level.

CHAPTER 6 MAPPING SECONDARY MYCELIAL ESTABLISHMENT FROM PAIRED SIBS OF P. velutina USING A DESTRUCTIVE SAMPLING TECHNIQUE

INTRODUCTION

Initial estimates of secondary mycelial establishment patterns in P. velutina could be made, with relative ease in many cases, by observing the distribution of abundantly clamped secondary phase hyphae within interacting primary mycelia. The boundary of this region was frequently marked by arcs of emergent cordlike secondary mycelial tufts, apparently formed at those points where further establishment/extension within the resident primary mycelium was prevented. An example in which secondary mycelium emerged as cords at the edge of the plate was chosen for a more detailed investigation on 14cm diameter Petri dishes of 2% MA. This was used to provide secondary mycelial maps based on a bioassay and to reveal whether the extent of such mycelia was limited within potentially larger resident primary mycelia.

MATERIALS AND METHODS

The pairing F25-14 x F25-18 (Fig. 5.4b) was set up using ca. 4mm diameter cylindrical inocula whose centres were placed 2cm apart on a straight line passing through the centre of the base of each dish. Twenty-one replicates were incubated at 20°C in darkness and three were destructively sampled after 6, 10, 12

days and thereafter at 2-day intervals until 20 days incubation had elapsed. Sample plugs of agar/mycelium (4mm diameter) were removed from positions within the interacting colonies determined by a 4mm square grid of lines boldly drawn on a white surface and positioned beneath the dish during sampling. Plugs were removed from the central square and then from alternate squares in a vertical and horizontal direction (Fig. 6.1a).

The criterion used in the detection of secondary mycelium within sample plugs was based on results of mycelial pairings in interaction class 2 (Table 5.16). An unrelated primary mycelial tester isolate was selected which gave an obvious mating-type compatible reaction with the chosen F25 sibs and an intense rejection zone against F25 14/18. A bioassay was performed in square repli-dishes (25 x 2cm square compartments in each dish) whose compartments each held 2% MA to a depth of ca. 4mm. They were inoculated with a sample plug/tester isolate pairing, as far apart as possible, and incubated at 20°C in darkness for 1 month (Fig. 6.1b). Control pairings of F25-14 and F25-18 were similarly incubated in 14cm diameter Petri dishes and sampled on day 20 from randomly chosen positions on the 4mm grid.

RESULTS

The bioassay produced easily-scored results, enabling maps to be drawn of secondary mycelial development within regions of interacting primary mycelia (Fig. 6.2). The shape of the mapped mycelium was basically similar within both resident colonies and was frequently encountered in P. velutina sib and

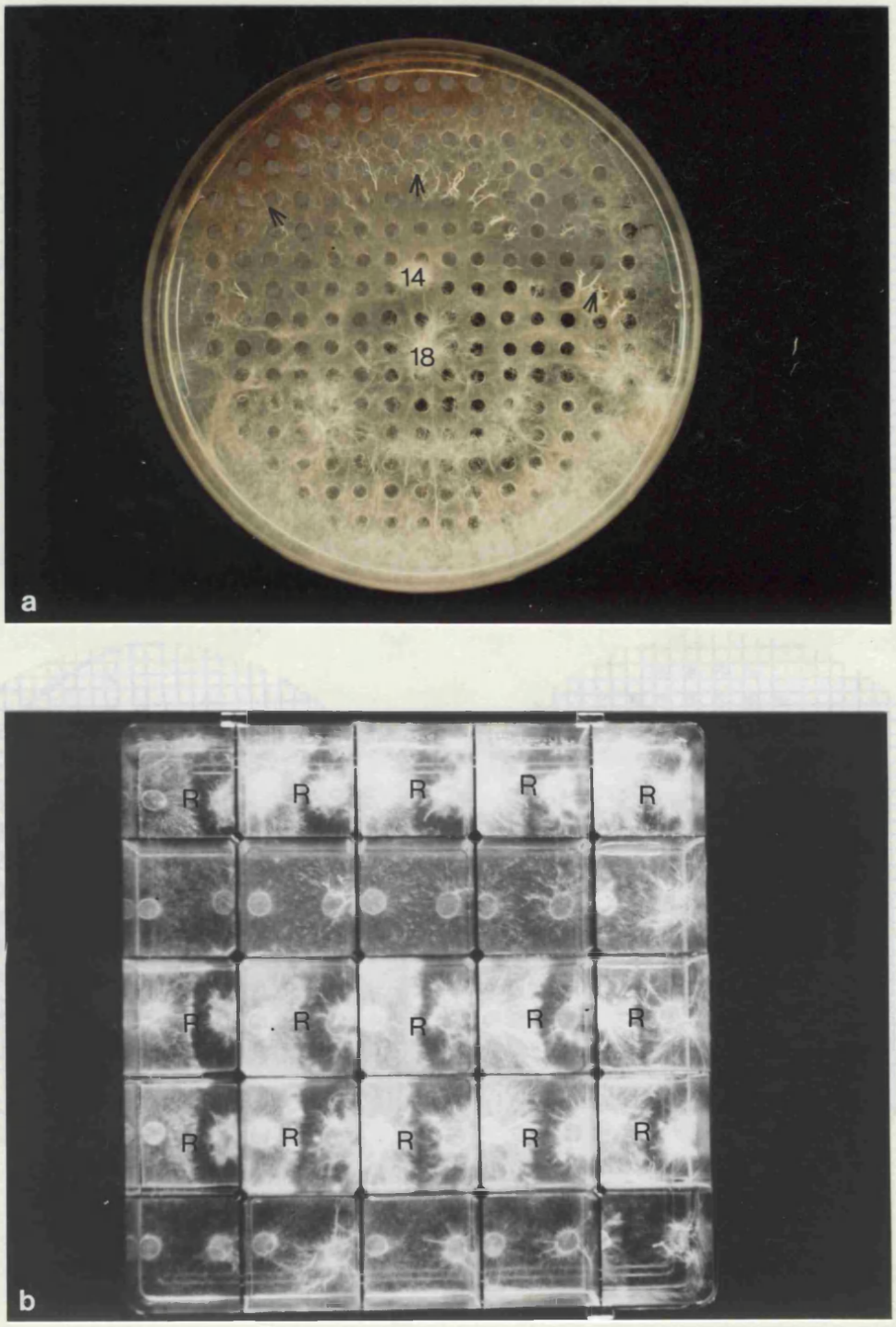


Fig. 6.1. Detection of secondary mycelium in paired sibs of P. velutina. (a) Mating-type compatible interaction between F25-14 and F25-18 after destructive plug sampling and further incubation. Arrows indicate the extent of visible secondary mycelium. (b) Repli-dish used for detecting secondary mycelium in plugs removed from (a). Each well contains such a plug paired against an unrelated primary mycelial tester inoculum. Secondary mycelium is indicated by rejection zones (R).

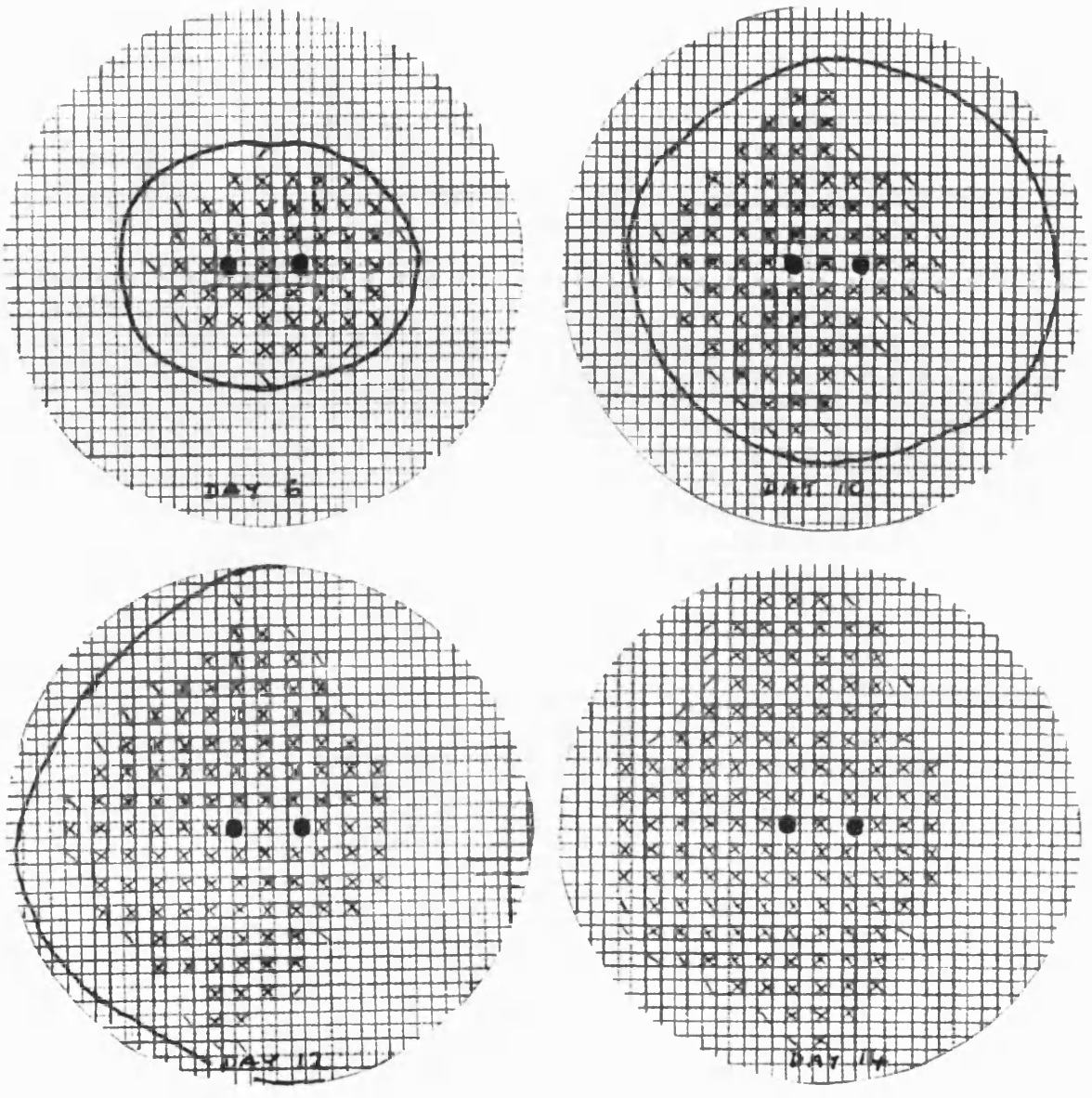


Fig. 6.2. Legend overleaf.

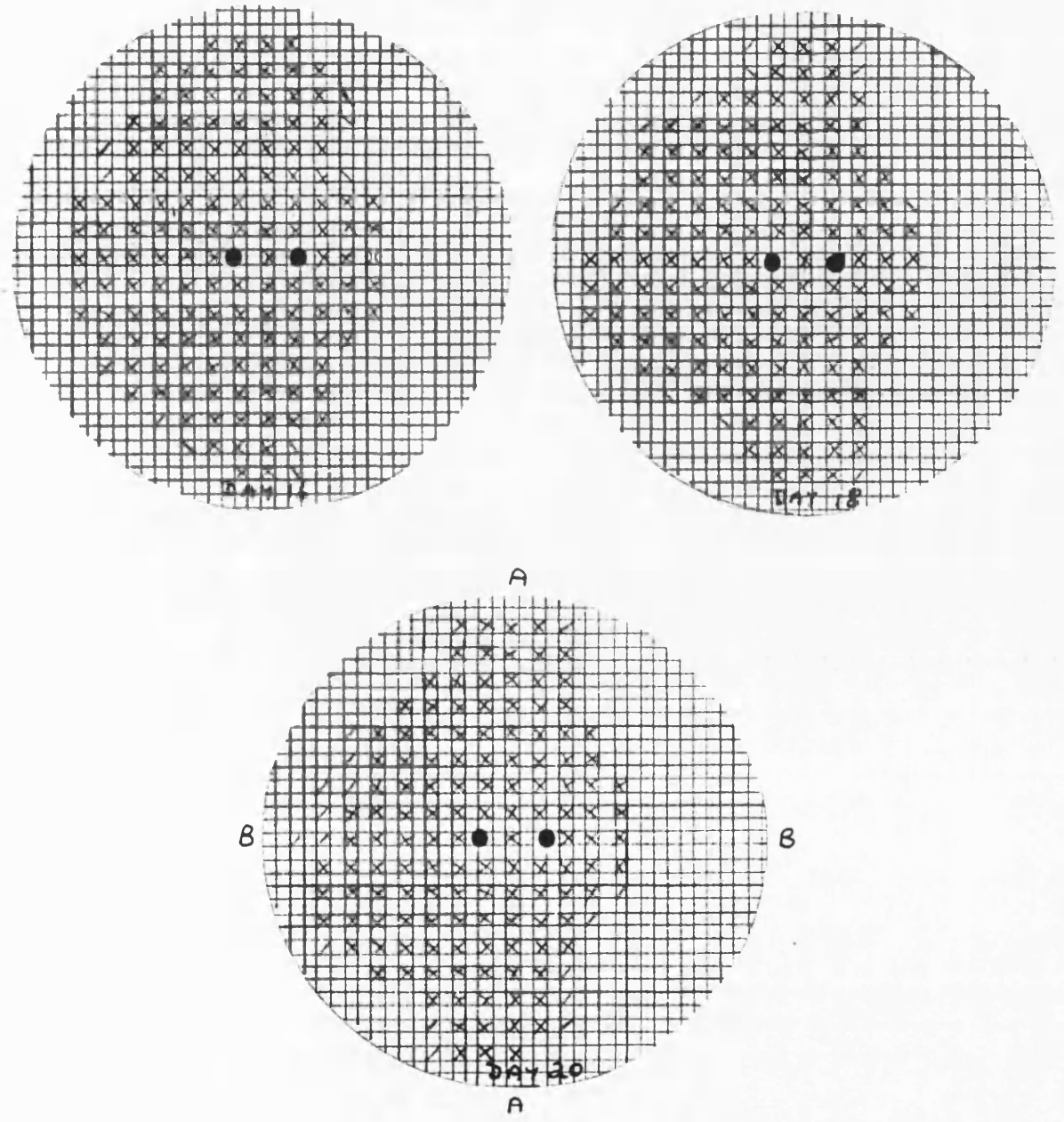


Fig. 6.2. Maps of secondary mycelium in paired sibs of P. velutina. Left and right inocula (●) are of F25-18 and F25-14 respectively. Solid lines indicate the mycelial margin 6-20 days after inoculation. (X,\,/) indicate secondary mycelial detection in 3,2,1 replicates respectively. A-A and B-B are reference points referred to in the text.

non-sib primary mycelial pairings in which secondary mycelial development was halted before reaching the edge of 9cm diameter dishes. In all these cases the maximum extent of secondary mycelium occurred in two perpendicular planes, under the experimental conditions, i.e. a major extension along the axis of the confrontation zone to the plate edge (A-A) and also back from the mid-point of the zone and perpendicular to it (B-B) (Fig. 6.2). The former direction of development seemed to be the most consistent, in that the edge of the plate was almost always reached. However, development in the pependicular direction often showed a remarkable unilateral character and early stasis within one of the paired colonies. In the analysed example, the progress of the secondary mycelium in the B-B direction within the region occupied by F25-14 was halted after ca. 10 days incubation, whereas it continued within the region occupied by F25-18 for ca. 6 more days.

DISCUSSION

In culture, P. velutina showed an interesting pattern of secondary mycelial establishment in that remnants of the primary phase often survived at sites distal to the confrontation zone. In the example analysed, primary phase radial extension continued until the plate margin was reached. Secondary mycelium was initially detected in progressively larger portions of the formerly primary mycelial regions, but its development was arrested at a different time for each. During this period, establishment of the secondary phase probably occurred as a result of combined mycelial penetration or intrusive growth and

the full range of hyphal interactions detected in primary mycelial and secondary/primary mycelial interactions (see Chapter 8). However, the lenticular shape and temporary expansion of secondary mycelium suggest that radially orientated nuclear migration was the most likely major underlying process in this establishment.

Control pairings of F25-18 had a higher radial extension rate than those of F25-14. On day 6 of the experimental pairings, secondary mycelium was established in a slightly larger portion of F25-18, although both colonies had similar radii. However, secondary mycelial expansion ceased earlier within F25-14 whose margin reached the edge of the dish before that of F25-18. Therefore, secondary mycelial establishment seemed to affect radial extension rate of component primary mycelia. Once physiological unity had occurred however, it was not possible in this study to discern the roles of genotypic interaction and resource reallocation in modifying hyphal growth at the colony margin.

If similar patterns occur in the field, the most likely route by which secondary mycelium could exit into regions of substratum which lack its precursive mycelia seems to be via the edge of the confrontation zone. The mode change to a more aggregated morphology which occurred at the secondary/primary mycelial interface may also facilitate penetration of and escape from a surrounding zone of primary phase remnants. Although occurring during a conspecific interaction between two developmental phases, this situation resembles a switch to an aggregated mycelial mode (cords or rhizomorphs) brought about by interspecific mycelial interactions as described by Rayner &

Webber (1984). In both cases, diffuse mycelial exploration is replaced by structures which are apparently adapted for extension in nutrient poor or otherwise hostile environments.

CHAPTER 7 USE OF MYCELIAL INTERACTIONS IN EXPERIMENTAL TAXONOMY WITHIN *Stereum*

INTRODUCTION

Once outcrossing had been demonstrated in *S. hirsutum*, it became possible to investigate the degree of overlap between the taxonomic and biological species concepts by studying the ability of primary mycelia to form secondary mycelia in simple pairing tests. In addition, as described by Coates *et al.* (1981), a readily scored multiple test was used. This involved four sib-related, mating-type incompatible primary mycelia which were inoculated equidistantly around the periphery of a 9cm diameter 2% MA plate which had a non-sib-related primary mycelial inoculum at its centre. In *S. hirsutum*, secondary mycelial formation always occurred such that the central primary mycelial region was superseded by four, or occasionally more, mutually heterogenically incompatible secondary mycelial sectors. However, there are two situations where this test could give false negative results: when all five isolates have a mating-type factor in common and when the central isolate blocks nuclear migration. The former situation can be detected if the test is repeated using peripheral isolates of another mating-type.

The present study also involved an investigation of the interactions between isolates from species regarded as distinct on morphological grounds, and between isolates from geographically separate locations and/or different woody species. Rayner & Turton (1982) investigated interfertility between *Stereum* spp. and concluded that their British collections of *S.*

hirsutum, S. gausapatum, S. rugosum, S. sanguinolentum and S. "rameale" were all intersterile. Attempts were made to confirm this work and to extend the survey to include S. subtomentosum and S. insignitum. In addition, the possibility of interaction types being associated with ecological specialization or geographical divergence was investigated using S. rugosum and S. hirsutum respectively.

MATERIALS AND METHODS

Tables 2.5, 2.9 respectively list the collection sites, codes and numbers of field and primary mycelial isolates of S. rugosum and S. hirsutum used in interspecific and intraspecific interactions. For both taxa, sibs were paired to assign mating-types and a representative of each mating-type was used in all possible conspecific non-sib pairings.

Finnish field isolates of S. hirsutum (K6,K8) were paired against each other and a range of conspecific primary mycelia from Finland and Britain. Both field isolates fruited in culture after 3 weeks routine incubation followed by 3 weeks on the laboratory bench at 15-25°C. Progeny sets of 10 sibs each were isolated from these basidiomata and subjected to the same procedure as wild basidiomatal progeny.

Confirmatory testing was applied to subcultures removed from either side of the pairings to detect the presence of secondary mycelium. Multiple testing for interfertility of primary mycelia was carried out within S. hirsutum and included those progeny of F2 which were obtained and originally used for this purpose by Coates et al. (1981).

Two mating-type or interaction group representatives were chosen for each species and used in interspecific interactions. These were paired in all such combinations and the multiple test was also used to assess interfertility of S. hirsutum, S. rugosum and S. gausapatum. For this purpose, mating-type representative isolates of S. gausapatum were selected from two interfertile progeny sets whose collection site details are shown in Table 2.10.

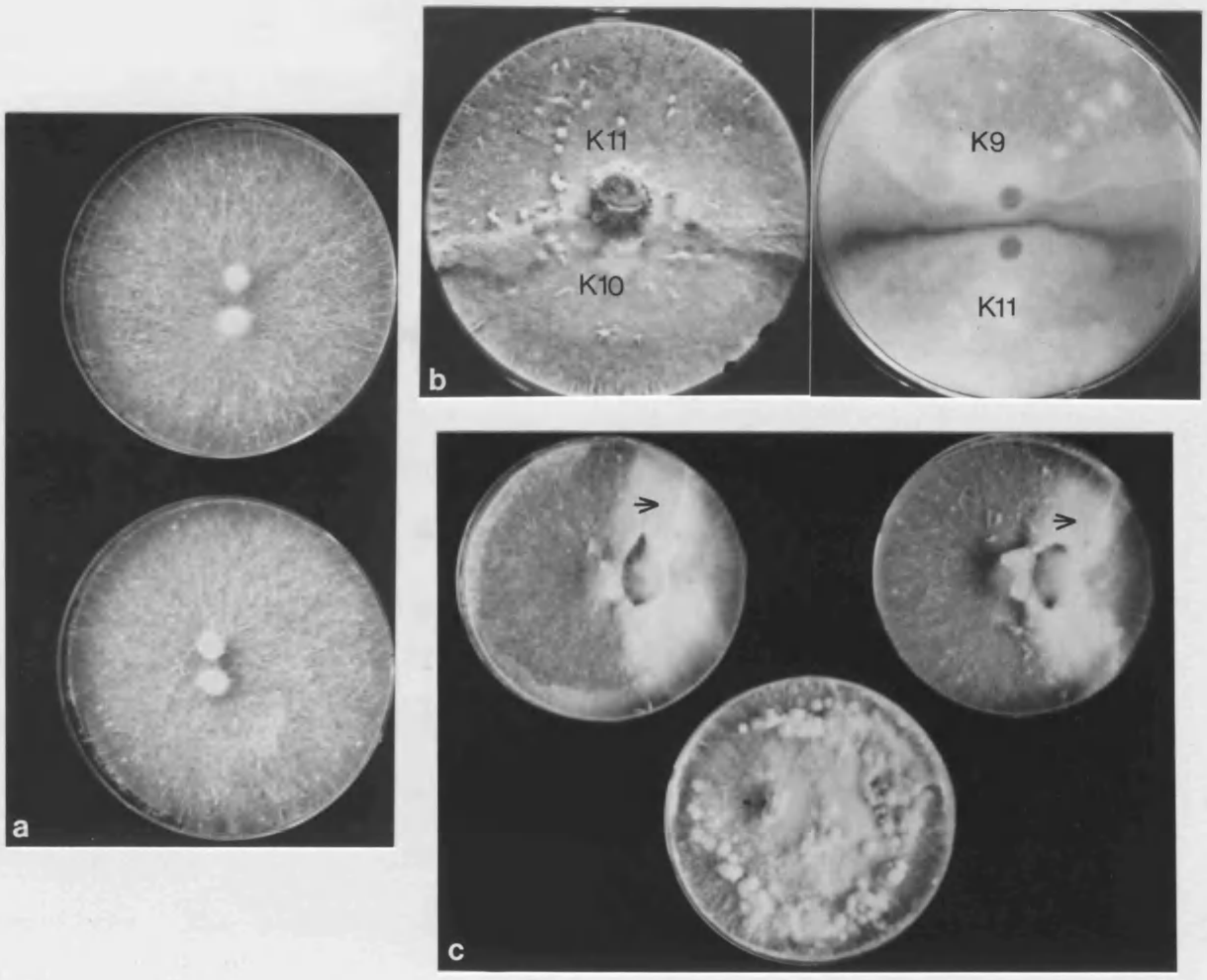
RESULTS

INTERACTIONS OF BRITISH AND FINNISH S. hirsutum ISOLATES

Finnish S. hirsutum primary mycelia rapidly developed confluent white aerial tufts which became irregularly pigmented buff to luteous, thereby resembling secondary mycelia of British isolates (Coates et al., 1981). Paired sibs of Finnish isolates (K6-K12) intermingled and were indistinguishable from control pairings (Fig. 7.1a). Those of K6 and K8 (derived from laboratory basidiomata) also intermingled with their respective parental field isolates.

Finnish non-sib interactions failed to produce any secondary mycelium; those of K7 and K9 intermingled, whereas the remaining primary mycelia remained morphologically distinct and sometimes separated by pale luteous to umber rejection zones, widest ($\leq$1cm) at their edges with concolorous aerial exudation (Fig. 7.1b,d; Table 7.1). Similar results occurred in pairings between field isolates and between field isolates and non-progeny primary mycelia. All Finnish collections of S. hirsutum were therefore shown to be non-outcrossing.

Fig. 7.1. Finnish non-outcrossing S. hirsutum mycelial interactions. (a) Intermingling in control pairing (above) of different parts of a single Finnish primary mycelium and a sib pairing (below). (b) Rejection in Finnish non-sib pairings (codes shown) viewed from above (left) and below (right). (c) Mycelial replacement (arrowed) of British outcrossing primary mycelium F1-4, seen in isolation (below), by Finnish non-outcrossing K7 sibs. (d) Subjectively assessed Finnish non-sib rejection intensity. (I) intermingling, (P) pigmented rejection, (D) isolates remain morphologically distinct without aerial mycelial bulking or discrete rejection zone.


K11
K10
b
K9
K11
a
c

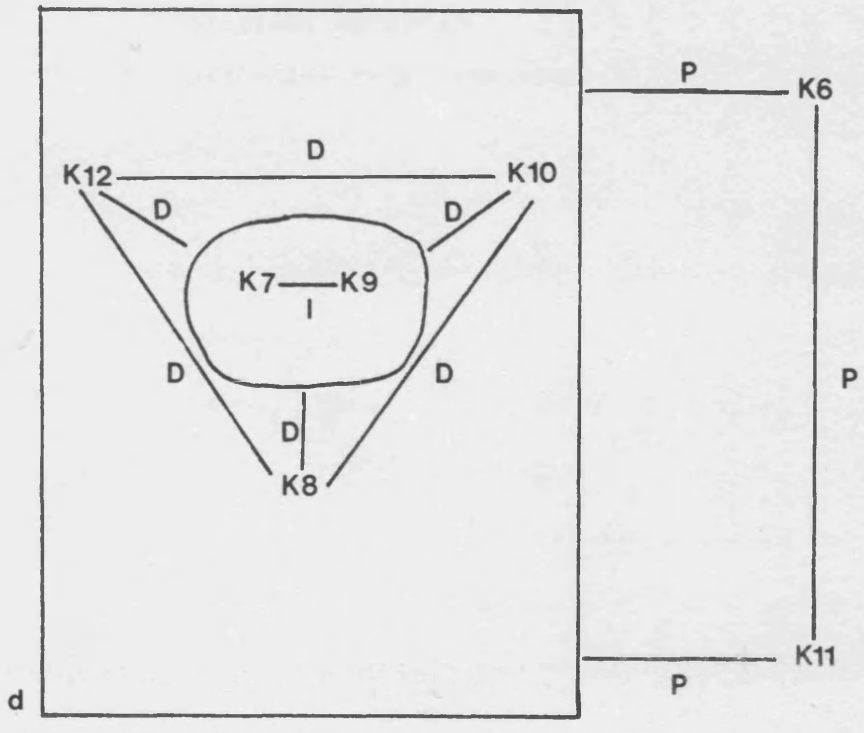

P
K6
D
K12
K10
D
D
K7
K9
I
D
D
P
D
K8
K11
P
d

Table 7.1. Non-sib interactions of Finnish S. hirsutum.

Isolate codes							
K6	K7	K9	K10	K12	K8	K11	
I	P	P	P	P	P	P	K6
	I	I	D	D	D	P	K7
		I	D	D	D	P	K9
			I	D	D	P	K10
				I	D	P	K12
					I	P	K8
						I	K11

I: intermingling

D: deadlock between morphologically distinct mycelia

P: pigmented rejection zone

Sib interactions of British F1 progeny are shown in Table 7.2 and those of F2 are given by Coates et al. (1981). Secondary mycelial production from certain paired British sibs and all British non-sibs in pairings and multiple tests were in accordance with an outcrossing strategy of unifactorial diaphoromixis.

No interaction between Finnish and British isolates resulted in secondary mycelial establishment. Instead, Finnish isolates usually overarched the confrontation zone and aerial mycelium gradually covered the British mycelia which was underlain by sienna to chestnut pigmentation (Fig. 7.1c). Subcultures from apparently replaced regions always generated the Finnish mycelial type which often fruited in the newly acquired territory. The identity of such fruiting mycelia was determined by pairing resultant progeny against the British and Finnish components of the original pairing.

INTERACTIONS BETWEEN BRITISH S. rugosum PRIMARY MYELIA FROM A RANGE OF SUBSTRATA

Table 7.3 shows the results of non-sib pairings of S. rugosum derived from basidiomata growing on a range of different substrata. All combinations yielded secondary mycelium by the criteria previously used (Chapter 5), with one exception, viz. that of GH1-4 against H1-1 (Fig. 7.2). That this was due to their having a common mating-type factor was supported by subsequent pairings between different GH1 and H1 isolates of the same mating-types as GH1-4 and H1-1. All such pairings resulted in a similar orange-sienna pigmented rejection zone obscured by a mound of white aerial mycelium.

Table 7.2. Sib interactions of S. hirsutum F1.

	Isolate codes														
	15	8	6	5	2	10	14	12	4	3	13	7	11	9	1	
	I	B	R	R	B	B	B	C	C	C	C	C	C	C	C	15
		I	B	R	B	B	R	C	C	C	C	C	C	C	C	8
			I	B	B	B	B	C	C	C	C	C	C	C	C	6
				I	B	B	B	C	C	C	C	C	C	C	C	5
					I	R	R	C	C	C	C	C	C	C	C	2
						I	R	C	C	C	C	C	C	C	C	10
							I	C	C	C	C	C	C	C	C	14
								I	B	B	R	B	B	B	B	12
									I	R	R	B	B	B	B	4
										I	R	B	R	B	B	3
											I	R	R	R	R	13
												I	R	B	R	7
													I	B	R	11
														I	B	9
															I	1

I: intermingling

B: bow-tie shaped zone of unstablized access migration

C: secondary mycelial establishment

R: rejection

Table 7.3. Non-sib interactions of *S. rugosum* involving a representative isolate from each mating-type class per basidioma.

Basidioma code											
Isolate codes											
H1	H2	RT12	GH1	GH2	U2	RO1	LG1	CH1	CR1	CR2	
5 1	14 10	1 6	1 4	1 2	6 4	1 2	1 6	1 2	1 4	1 3	
I C	C C	C C	C C	C C	C C	C C	C C	C C^1	C C	C C	H1-5
I	C C	C C	C P	C C	C C	C C	C C	C C	C C	C C	H1-1
	I C	C C	C C_2^1	C_2C_2	C C	C C	C^1C_2	C^1C	C^1C	C C	H2-14
	I	C C	C C^1	C C	C C	C C	C C	C^1C^1	C C	C C	H2-10
		I C	C C^1	C C_2^1	C C	C C	C C	C C	C C	C C	RT12-1
		I	C C	C C	C C	C C	C^1C	C C	C C	C C	RT12-6
			I C	C C	C C_2	C C	C C	C C	C C	C C	GH1-1
			I	C C	C C	C C	C_2^1C	C C	C C	C C	GH1-4
				I C	C C_2	C C	C C	$C_2^1C_2$	C C	C C	GH2-1
				I	C C_2	C C	C C	C C	C C	C C	GH2-2
					I C	C C	C C	C C	C C	C C	U2-6
					I	C C_2	C C	$C_2^1C^1$	$C_2^1C_2^1$	C C	U2-4
						I C	C C	C_2C	C C	C C	RO1-1
						I	C C	C C	C C	C C	RO1-2
							I C	$C_2^1C_2^1$	$C_2^1C_2^1$	C^1C	LG1-1
							I	C C	C C	C C	LG1-6
								I C	C C	C C	CH1-1
								I	C C	C C	CH1-2
									I C	C C	CR1-1
									I	C C	CR1-4
										I C	CR2-1
										I	CR2-3

I: intermingling

C: secondary mycelial establishment

C^1: C with mottled regions of mycelium

C_2: C with pigmented patches of mycelium

P: pigmented rejection zone

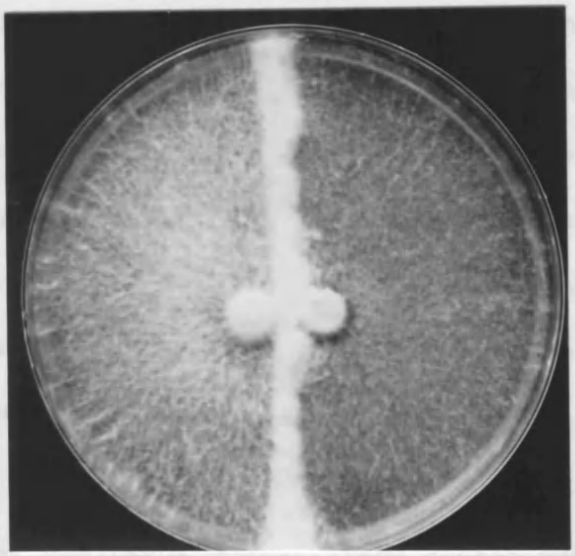


Fig. 7.2. Rejection between mating-type incompatible non-sibs in S. rugosum.

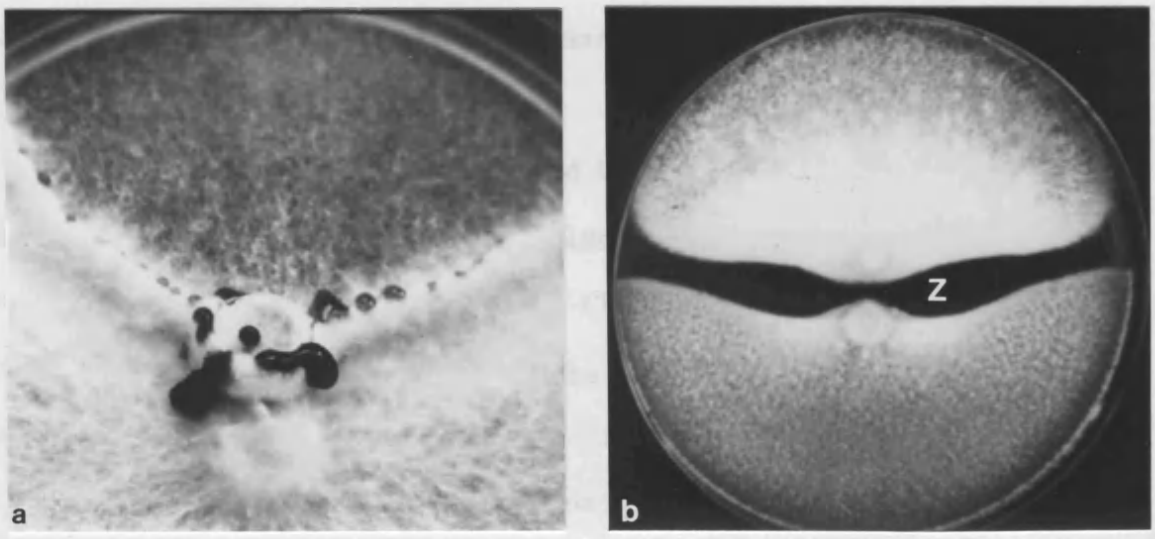


Fig. 7.3. Interspecific primary mycelial interactions. (a) Intense rejection with droplet exudation between S. rugosum (above) and S. gausapatum (below). (b) Mutually inhibited mycelial margins of S. "rameale" (above) and S. subtomentosum (below) bordering a zone (Z) of uninhabited medium.

Mating-type compatible interactions between the 21 different mating-type specificities represented in this study often involved a persistent hyaline confrontation zone which was most pronounced when the accompanying aerial tufts were relatively sparsely distributed. When present in greater numbers, there was often a marked unilateral tuft distribution with respect to the confrontation zone. Several interactions (see Table 7.3) involved the production of mottled regions extending from the confrontation zone which contained hyphae which had presumably undergone unstabilized access migration. Some of these regions also developed irregular submerged patches of orange to scarlet pigmentation, particularly along the edge distal to the confrontation zone.

INTERSPECIFIC PRIMARY MYCELIAL INTERACTIONS

Intersterility was confirmed between S. hirsutum, S. gausapatum, S. rugosum, S. sanguinolentum and S. "rameale" as previously shown by Rayner & Turton (1982). The present results indicate that S. subtomentosum and S. insignitum can also be added to this list.

Generally, those interactions involving producers of abundant aerial mycelium, i.e. S. hirsutum and S. gausapatum, were characterized by the formation of bulked ridges of aerial growth at and often overarching the confrontation zone. This could also be accompanied by pigmented aerial droplet exudation (Fig. 7.3a). The remaining species interacted to produce discrete pigmented zones in which aerial mycelium was absent or greatly reduced, but which formed low flanking aerial mounds.

The interaction of S. subtomentosum against S. "rameale" was notable in the development of a wide (≤1cm) pure yellow to luteous intervening zone from which hyphae were excluded (Fig. 7.3b). Hyphal apices on both sides of this non-fusion response were uniformly narrow, much branched and, based on the vacuolated and granular texture of their cytoplasm, were undergoing apparently mutually induced lysis.

DISCUSSION

INTERSPECIFIC INTERACTIONS AND S. hirsutum POPULATIONS IN BRITAIN AND FINLAND

A complete lack of interfertility between British primary mycelia of Stereum spp. is in accordance with the view that biological and taxonomic species concepts often coincide. If there was a barrier preventing genetic exchange between two groups which were once a hologamodeme then, a priori, permanent discontinuities in variation may develop between the two new hologamodemes rendering each a recognizable taxonomic entity. If genetic exchange persisted, the probability of such discontinuities developing would be greatly reduced.

It is suggested that the full potential of interfertility testing should be realized. Hitherto, the majority of studies have reported the results of such tests as positive or negative, often depending entirely on the presence or absence of clamp connections. It is postulated that many, if not all, of the stages in the interaction sequence initiated by close proximity of mycelia could act as fertility barriers. It would be more

informative therefore to record the stage of metabolic intimacy achieved. For example, intersterility may result from mycelia interacting without hyphal contact, by hyphal interference (see p.259), post-fusion rejection, temporary override of rejection forming a heterokaryon by unstabilized access migration, or by establishment of a sterile secondary mycelium.

Collections of the taxa S. sanguinolentum, S. "rameale" and S. subtomentosum have so far revealed a consistent non-outcrossing strategy which necessarily implies that both the biological species concept and interfertility testing on which it is based are inappropriate. If interaction groups could be resolved into clones then each could be called a genodeme to distinguish the situation from one in which a taxonomic species is partitioned into several hologamodemes, (sibling species), each of which may be a nascent taxon.

Perhaps the most important finding of this preliminary investigation into breeding and non-breeding units was that of British outcrossing and Finnish non-outcrossing populations within S. hirsutum. Although the Finnish collections were limited in number, nevertheless their population structure of genodemes shared several features with that of S. sanguinolentum and S. "rameale" (Chapter 3) as follows:

i) Members of an interaction group (K7 and K9) were collected from different sites.

ii) Isolates which interacted weakly with this interaction group could be derived from the same site (K10) or different sites (K8 and K12).

iii) Isolates producing strongly pigmented interaction zones could be derived from the same site (K11

against K9 or K10) or different sites (K6 against K11).

Therefore, the speculated mode of origin of a non-outcrossing breeding strategy from an ancestral outcrossing system advocated in Chapter 3 seems equally applicable to the Finnish isolates. Indeed, the possibility of comparing outcrossing primary, outcrossing secondary and non-outcrossing primary mycelia has provided some evidence supporting the role of spontaneous functional heterokaryosis in the absence of two dissimilar genomes. It is acknowledged that *per se* the morphological change accompanying primary/secondary transitions in outcrossing *S. hirsutum* is not of sufficient magnitude for reliable identification of the two phases (Coates *et al.*, 1981). Nevertheless, the cultural characteristics of Finnish primary mycelia more closely resembled those of British secondary rather than primary mycelia. Furthermore, Finnish isolates fruited regularly in plate culture, an event which has only occurred, albeit with poor and relatively tardy results, in the secondary phase of British *S. hirsutum* (Coates & Rayner, 1985a,b). Another secondary phase feature shown by the non-outcrossing primary phase was the apparent inability to receive genetically dissimilar nuclei from conspecific outcrossing primary mycelia. However, unlike true secondary mycelium, Finnish isolates also seemed incapable of donating nuclei to outcrossing primary mycelia.

If the non-outcrossing strategy arose through the action of a cytoplasmically transmissible mode-switching factor as suggested in Chapter 3, then the interactive behaviour of Finnish primary mycelia further suggests that since the loss of

outcrossing ability, the factor has ceased to be mobile. Fruiting primary mycelia transformed by the mobile factor produced some progeny whose interactive behaviour indicated that their mating-type had switched to that of a complementary type. The specificity thus acquired was also shown to be present among the sibs of the fruiting isolate. This led Coates & Rayner (1985b) to suggest that the transmissible factor concerned consists of that part of a mating-type factor which controls access migration and possibily stabilization.

There is increasing evidence for mating-type switching in the Ascomycotina, e.g. in Chromocrea spinulosa (Mathieson, 1952) and Sclerotinia trifoliorum (Uhm & Fujii, 1983), pseudoselfing in O. ulmi (Brasier & Gibbs, 1975) and, in particular, the cassette system in Saccharomyces cerevisiae (Hicks, Strathern & Klar, 1979; reviewed by Yanagishima, 1984). However, mutational studies in S. commune and C. cinereus have failed to reveal "silent" mating-type loci leading Ullrich, Novotny & Specht (1985) to conclude that they are absent in the Basidiomycotina. Clearly further work is required within genera such as Stereum to resolve this issue and, if appropriate, to relate the cassette system to multiallelic and non-outcrossing breeding strategies in the Basidiomycotina.

S. rugosum AND ECOLOGICAL SPECIALIZATION

Ecological specialization has been associated with partial interfertility barriers and hence speciation within taxonomic species which are currently sympatric, e.g. N. American Hyphoderma mutatum (Peniophora mutata) on poplars (Populus) or

other broad-leaved trees (McKeen, 1952), N. American Hirneola (Auricularia) auricula-judae on "coniferous" or "deciduous" (sic) substrata (Duncan & Macdonald, 1967) and the numerous physiological races of rusts (Burnett, 1983). Boddy & Rayner (1983) revealed a sterility barrier (post-fusion rejection) between Phlebia rufa and P. radiata and suggested that this too was associated with differences in ecological strategy. Indeed, Duncan (1972) stated that ecological isolation was undoubtedly the main force promoting speciation in the Basidiomycotina.

In contrast, although the present investigation revealed degrees of impeded mating competence between S. rugosum non-sibs, there was no evidence for an obvious ecological factor. It seems more likely that mating-type compatible interactions involving intense hyaline confrontation zones, patches of mottled mycelial morphology and pigmentation are in accordance with the fluctuating acceptance/rejection balance model described on pages 39-41. That greater genetic dissimilarity existed between non-sibs than between sibs was supported by the subjectively assessed greater strength of rejection between mating-type incompatible non-sibs compared to that between sibs. Indeed, between the former this more closely resembled that between field isolates or laboratory-synthesized secondary mycelia.

PART II

HYPHAL INTERACTIONS

CHAPTER 8 HYPHAL INTERACTIONS WITHIN P. velutina

INTRODUCTION

Reviewers of hyphal fusion studies, e.g. Burnett (1976), Carlile & Gooday (1978), Aylmore & Todd (1984a), Gregory (1984) and Nguyen & Niederpruem (1984) have recognized the importance of Buller's (1931) outstanding microscopic observations of living hyphal responses. In his own review of the subject, Buller (1933) emphasized the fundamental distinction that should be made between hyphal fusion, which establishes a protoplasmic link between hyphae and hyphal contact which does not. He applied the term hyphal adhesion to the intermediate situation in which the intact intervening wall layers are not so readily separable and yet fusion still does not occur.

Within the Basidiomycotina, Buller (1931, 1933) recognized two main types of fusion, i.e. having either vegetative or sexual consequences, which essentially facilitated nutrient/stimulus or nuclear movements respectively. Vegetative fusions converted a radiate mycelium into a network for efficient nutrient conduction to reproductive or perennating structures, thereby enabling regions of mechanical damage to be bypassed. Sexual fusions allowed mating-type compatible nuclei to associate in conjugate pairs and facilitated nuclear migration by increasing the number of available routes. In addition, Buller (1931) envisaged a role for hyphal fusion in fungal social organization which became known as the unit mycelium concept (see p. 31). Interestingly, an example of such co-operation had been

provided over 50 years earlier by the probable discoverers of hyphal fusion, Tulasne & Tulasne (1931). Via this English translation, the brothers reported that anastomosing germlings of the same species became united by linear isthmuses to form mycelium. However, they also noted that at least some of the spores used in this work were asexual, which suggests that they may have been genetically identical and therefore not representative of hyphal fusion in general.

Buller (1933) also considered the spatiotemporal relationships of hyphal fusions. He noted that peripheral young hyphae may grow in close proximity or cross over with no tendency to fuse, which suggested that fusion may be associated with nutrient exhaustion in older parts of the mycelium. However, Buller (1933) also noted that hyphal fusion seemed to prevent further fusion in the immediate vicinity. Moreover, once a certain number of fusions per unit length of almost parallel hyphae had been reached, increasing with decreasing interhyphal distance, further fusion was apparently prevented.

Attention was then directed away from the study of living hyphal fusions and with a few exceptions, e.g. nuclear exchange in mating-type compatible interactions in C. versicolor (Lange, 1966) and Clitocybe truncicola (Bistis, 1970), this neglect persisted until recent phase contrast and electron microscope time course studies of C. versicolor and S. commune. Aylmore & Todd (1984a) usefully classified hyphal fusion on the basis of genetic similarity between the two interacting hyphae. Self fusion distinguished those cases in which both participants were genetically identical from non-self fusions of genetically different hyphae.

In C. versicolor, self fusion of secondary phase hyphae (dikaryons) resulted in macroscopic intermingling in plate culture. By contrast, the confrontation zone of corresponding non-self interactions remained obvious as a narrow pigmented zone of relatively sparse mycelium containing spindle cells, i.e. enlarged highly-refractile lengths of hyphae, (Rayner & Todd, 1979; Todd & Rayner, 1980). However, Aylmore & Todd's (1984a) observations of living C. versicolor hyphae indicated that self and non-self fusion of secondary mycelia, self fusions of primary mycelia and fully mating-type compatible secondary/primary (di-mon) fusions could all share a common fate. This was called the nuclear replacement reaction and similar observations had previously been made in Coprinus by Bensaude (1918; cited by Noble, 1937) and in Typhula trifolii by Noble (1937). Briefly, it involved migration via the fusion bridge of a donor nucleus, or pair of conjugate nuclei, into a recipient hypha whose resident nucleus or nuclei degenerated. Nuclear division of the donor nuclei, or nucleus in monokaryon self-fusions, was followed by septation which re-established the former nuclear condition of the donor in both compartments thus formed. Non-self monokaryon interactions were limited to four mating-type compatible encounters, two of which initiated nuclear replacement and two initiated septal erosion and nuclear migration.

Self fusion of S. commune dikaryons usually resulted in nuclear replacement, whilst that of monokaryons had variable results which included persistence of a dikaryotic fusion compartment and disintegration of one of the donor's daughter nuclei (Nguyen & Niederpruem, 1984; Todd & Aylmore, 1985). Non-self fusions of S. commune monokaryons were observed in two

mating-type compatible combinations and resulted in septal erosion, nuclear migration and formation of multinucleate hyphal apices (Niederpruem, 1980a). Further development of the latter involved progressive reduction in nuclear numbers until a stable dikaryotic state was reached (Niederpruem, 1980b). Fully mating-type compatible di-mon fusions were observed by Nguyen & Niederpruem (1984) in which dikaryons comprising the monokaryon's genotype could be established with or without septal erosion and nuclear migration from the fusion compartment.

MATERIALS AND METHODS

Homokaryotic primary mycelia were selected from progeny sets listed in Table 2.6 and heterokaryotic secondary mycelia were selected from corresponding field isolates or synthesized from paired mating-type compatible homokaryons. Mycelia were grown on cellophane membranes which had been soaked in 50% v/v glacial acetic acid and absolute ethanol for 20 min, washed, then autoclaved separately in distilled water at 121°C for 15 min. Each membrane was then applied to the surface of a 9cm diameter 0.02% w/v MA plate, inoculated with one or two isolates for self and non-self fusion studies respectively and incubated in darkness at 22°C for a minimum of 3 days.

A square of cellophane ($\leq 10mm^2$) bearing hyphal apices of the inoculated isolate(s) was then removed and loaded into a microculture chamber (for details of chamber preparation and inoculation, see Aylmore & Todd, 1984b). After overnight incubation at 22°C in darkness, the chambers were transferred to a Wild M20 microscope and observed at 22-25°C using phase

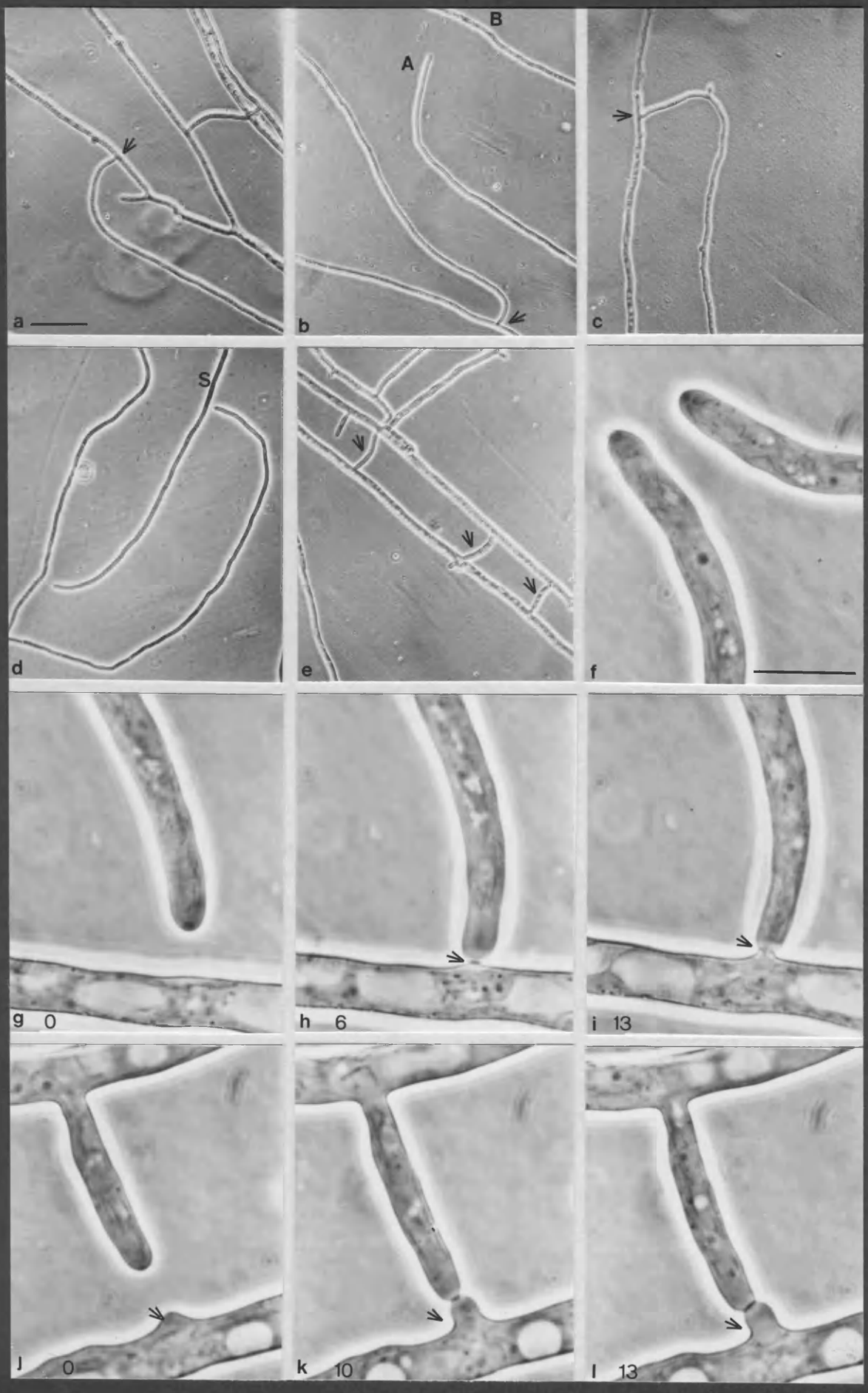
contrast and dark ground optics over a period of 48h. A total of 30 full sequences of self fusion, 20 of non-self fusion between heterokaryons, 20 of fusion between mating-type compatible homokaryons, 11 of fusion between mating-type incompatible homokaryons and numerous partial sequences were documented by notes, drawings and photography. Photomicrographs were taken on Kodak Technical Pan 2415 film rated at 50ASA and developed in Acutol (Paterson Products) or Technidol (Kodak).

RESULTS

EVENTS PRECEDING HYPHAL FUSION

Self and non-self fusions were preceded by similar hyphal responses. Main hyphal apices often showed long-range curvature (homing), over a maximum distance of 250μm, which was apparently directed towards specific receptive sites in the lateral wall of neighbouring hyphae (Fig. 8.1 a-d). Occasionally, this involved the longitudinal hyphal axis turning through an angle exceeding 90°, particularly when curvature was initiated slightly ahead of a receptive site on an almost parallel hypha (Fig. 8.1c). Convergent apices of main hyphae repelled each other (Fig. 8.1f) and receptive sites generally only occurred in a region extending backwards from the rear of the apical compartment. However, older compartments of main hyphae with extensive vacuolation, less dense cytoplasm and hence more prominent nuclei were also unreceptive to fusion. Fusions involving main hyphal apices commonly occurred without induction of a growing point from the receptive site, i.e. they were tip-to-side fusions, otherwise

Fig. 8.1. Events preceding hyphal fusion in P. velutina. (a) Self fusion of heterokaryotic main hyphal apex to recipient site (arrowed) on lateral wall following intra-mycelial homing. (b) Homing of main hyphal apex (homokaryon A) to lateral wall of mating-type compatible homokaryon (B) and non-self fusion (arrowed) between hyphal apex of B and lateral wall of A. (c) Self fusion of heterokaryotic apex to recipient site (arrowed) following curvature of the former through >90° during homing. (d) Reciprocal homing between mating-type compatible homokaryons. Septum (S) delimits an apical compartment whose apex and lateral wall were both subsequently involved in non-self fusion. (e) Scalariform H-bridges (arrowed) formed by self fusions between heterokaryotic main hyphae. (f) Mutual repulsion between previously convergent heterokaryotic main hyphae within a single mycelium. (g-i) Homing and self fusion of heterokaryotic main hyphal apex. Note the slight lateral wall deformation (arrowed) at the recipient site just before (h) and after (i) contact. (j-l) Initiation of H-bridge in a heterokaryotic mycelium. Self fusion followed tip-to-tip appression involving induction of a hyphal tip (arrowed) from a recipient site in the lateral wall. Bar markers represent 100μm (a-e) and 10μm (f-l) respectively. Time (min) is indicated in the lower left-hand corner of (g)-(i) and (j)-(l).


a
b
A
B
c
d
S
e
f
g 0
h 6
i 13
j 0
k 10
l 13

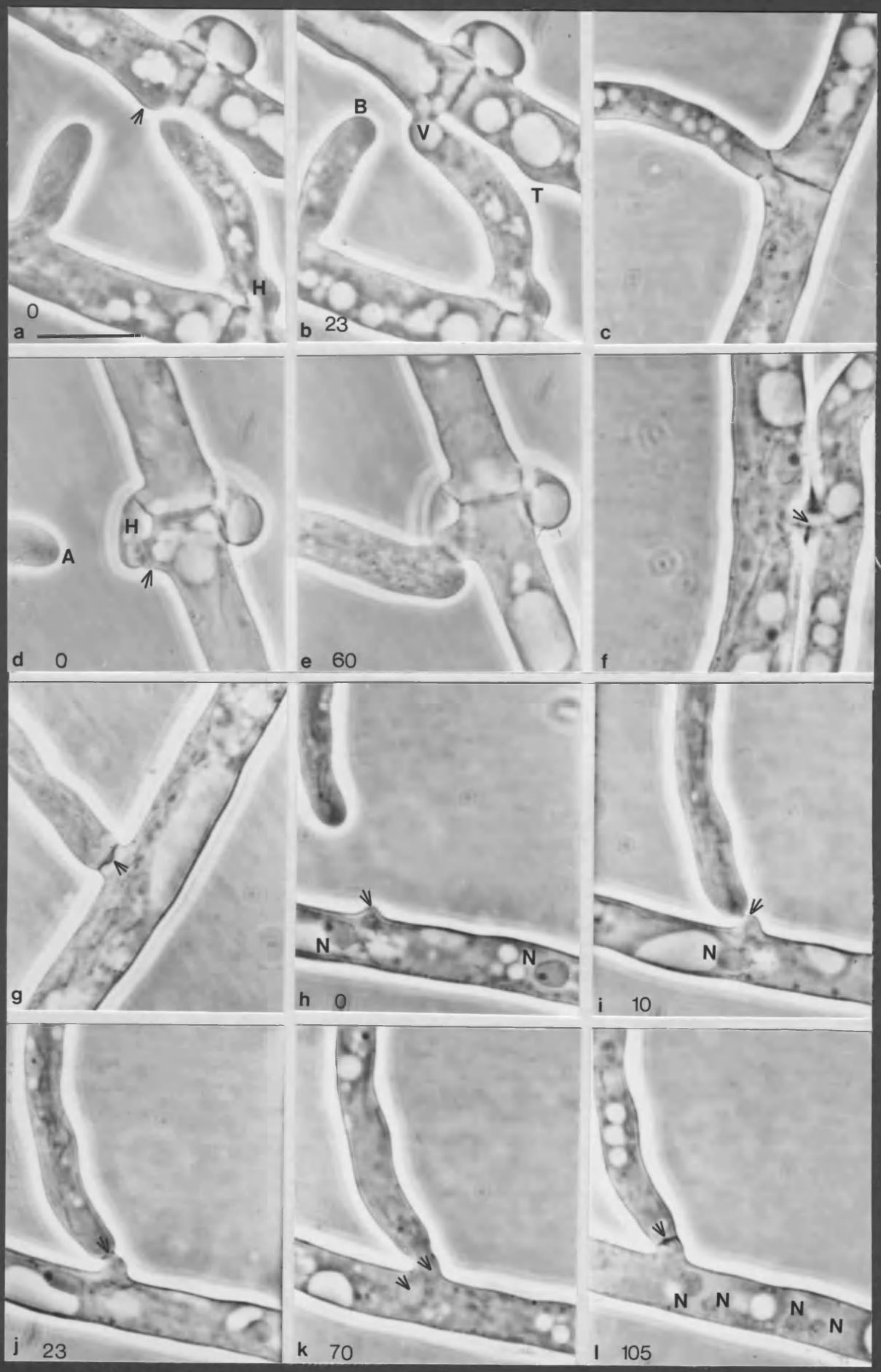
there was minimal lateral wall distortion immediately before, or just after contact (Fig.8.1j-l).

Short-range fusions, resulting in the formation of H-bridges between hyphae were also common (Fig. 8.1e), but usually involved less marked curvature and were preceded by development of a growing point (peg) by the receptive hypha (Fig. 8.1g-i). When several pegs were initiated close together between neighbouring hyphae, development of a few usually halted when they were mere lateral bulges. Extension of the remainder continued or stopped and resumed until hyphal fusion established a single H-bridge when all residual peg extension was halted except near septa.

In the example shown in Fig. 8.2a,b, two branch hyphae originated from contiguous compartments and homed towards the same receptive site. This involved one of them growing past a peg which had ceased extension and was apparently no longer receptive to fusion. Fusions frequently occurred near and on the subapical (Fig. 8.2c,d), apical (Fig. 8.2a,b) or both sides of septa. A single apex rarely simultaneously fused with the compartments on either side of a septum (Fig. 8.2e) and presumably fusions in this region were associated with sites of potential hook cell formation and fusion.

Apical bodies (Spitzenkörper) were only prominent in growing hyphal tips and in several cases it was possible to observe their displacement and alignment with a receptive site before curvature towards and fusion with such sites (Fig. 8.3a-c). By contrast, appression fusions did not seem to involve apices, but merely resulted from the opening of a pore in the lateral walls of two hyphae that had been in contact for an extended period (Fig. 8.2f).

Fig. 8.2. Hyphal fusion in relation to septa and events after self fusion in P. velutina. (a,b) Self fusion within a homokaryon establishing an H-bridge between a hook cell (H) and lateral bulge (arrowed) at the front of a septum. Note the slight homing curvature of neighbouring tip (B) just before it ceased extension and the constricted vacuole (V) passing through the fusion pore. Slight transient tip induction (T) occurred to the rear of the septum, but development ceased before (a). (c) Non-self fusion between heterokaryons simultaneously involving one apical and two recipient intercalary compartments. (d,e) Homing and non-self fusion between a heterokaryotic apex (A) and the rear of a septum. The adjacent hook cell (H) had induced a growing point (arrowed), but fusion to form a clamp connection had not occurred. (f) Non-self appression fusion (arrowed) between two heterokaryons. (g) Interfacial bulge (arrowed) between homokaryotic hyphae of a single mycelium which had been in contact for 30 min. Self fusion occurred 4 min later. (h-l) Nuclear division and septation during self fusion in a heterokaryon. Arrows indicate (h) pre-fusion hyphal tip induction, (i) hyphal contact, (j) site of fusion pore opening, (k) pair of dividing nuclei and (l) dolipore septum across the fusion pore. Nuclei (N) are shown before (h)-(i) and after (l) division. Bar marker represents 10μm. Time (min) is indicated in the lower left-hand corner of (a),(b),(d),(e), and (h)-(l).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j 23
k 70
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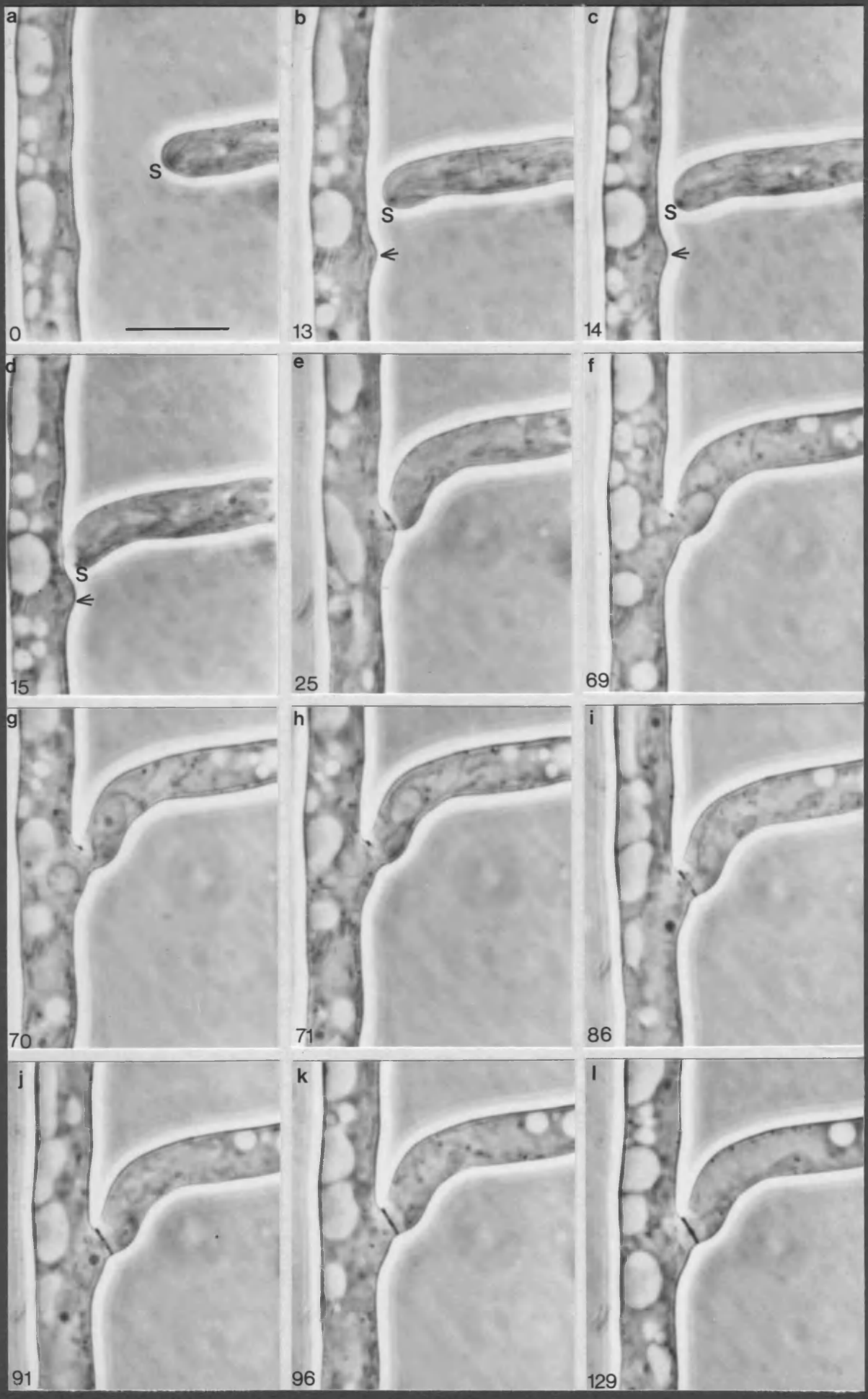
Fig. 8.3. Self fusion sequence in a P. velutina heterokaryon showing displacement of Spitzenkörper (S) and homing of hyphal apex (a-d) to induced tip (arrowed) in lateral wall of recipient compartment. Opening of the fusion pore (e) preceded nuclear division (f-h) and dolipore septal synthesis (i-l), both occurring at the site of fusion. See Fig. 8.4 for interpretive diagrams of nuclear behaviour in the pore region. Bar marker represents 10μm. Time (min) is indicated in the lower left-hand corners.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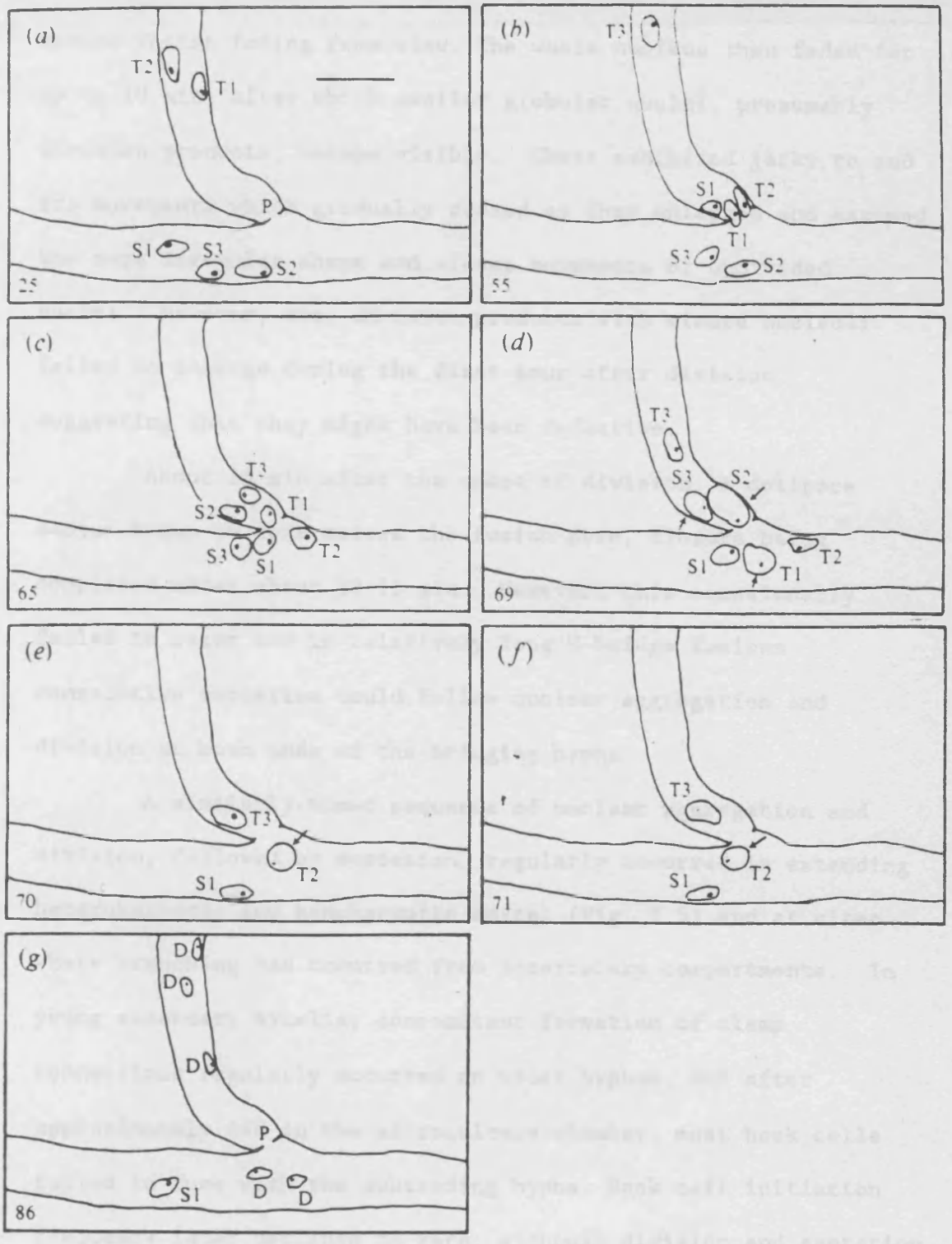
EVENTS AT AND AFTER SELF FUSION

These are illustrated in Fig. 8.2h-l, Fig. 8.3 and Fig. 8.4. After contact, a period (usually 5-20 min) of interfacial expansion preceded the formation of a fusion pore. Pore opening could only be reliably detected by observing the passage of organelles. However, when the contact-to-opening time was relatively protracted, it was heralded by an interfacial bulge into one of the participating compartments. In tip-to-side and tip-to-peg fusions this was usually directed into the latter compartment types (Fig. 8.2g) and was apparently produced by a combination of a turgor pressure difference and progressive enzymatic thinning of the interfacial walls.

The fusion pore usually enlarged for ca. 15 min until it occupied virtually the entire contact zone, although a visible interfacial rim sometimes persisted (See Aylmore & Todd, 1986a). Filamentous mitochondria were observed traversing the pore, whilst vacuoles and cytoplasmic granules showed bidirectional movements, usually after an initial unilateral phase. In tip-to-side and tip-to-peg fusions, initial organelle movement was into the latter compartment type in accord with the inferred turgor pressure gradient.

Nuclear behaviour was complex with initial movements of one or more from one hypha into the other being followed by a general aggregation of four to six in the vicinity of the fusion pore. When the pore had been open for about 40-45 min, some or all of the aggregate entered a division cycle. Starting within a 5 min period, the nuclear membrane dilated whilst the enclosed nucleolus contracted and seemed to move with rapid Brownian

Fig. 8.4. Diagram illustrating the sequence of events during the self fusion shown in Fig. 8.3. T1-T3 and S1-S3 are nuclei derived from the apical and recipient compartments respectively. (a) Pore (P) visibly open. (b,c) Aggregation of apical and recipient compartment nuclei in pore region. (d-f) Arrowed nuclei in early stages of division. (g) Septum forming across pore (P); note daughter nuclei (D). Bar marker represents 10μm. Time (min) is indicated in the lower left-hand corners such that time 0 corresponds to that of Fig. 8.3(a).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(d)
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(e)
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(f)
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(g)
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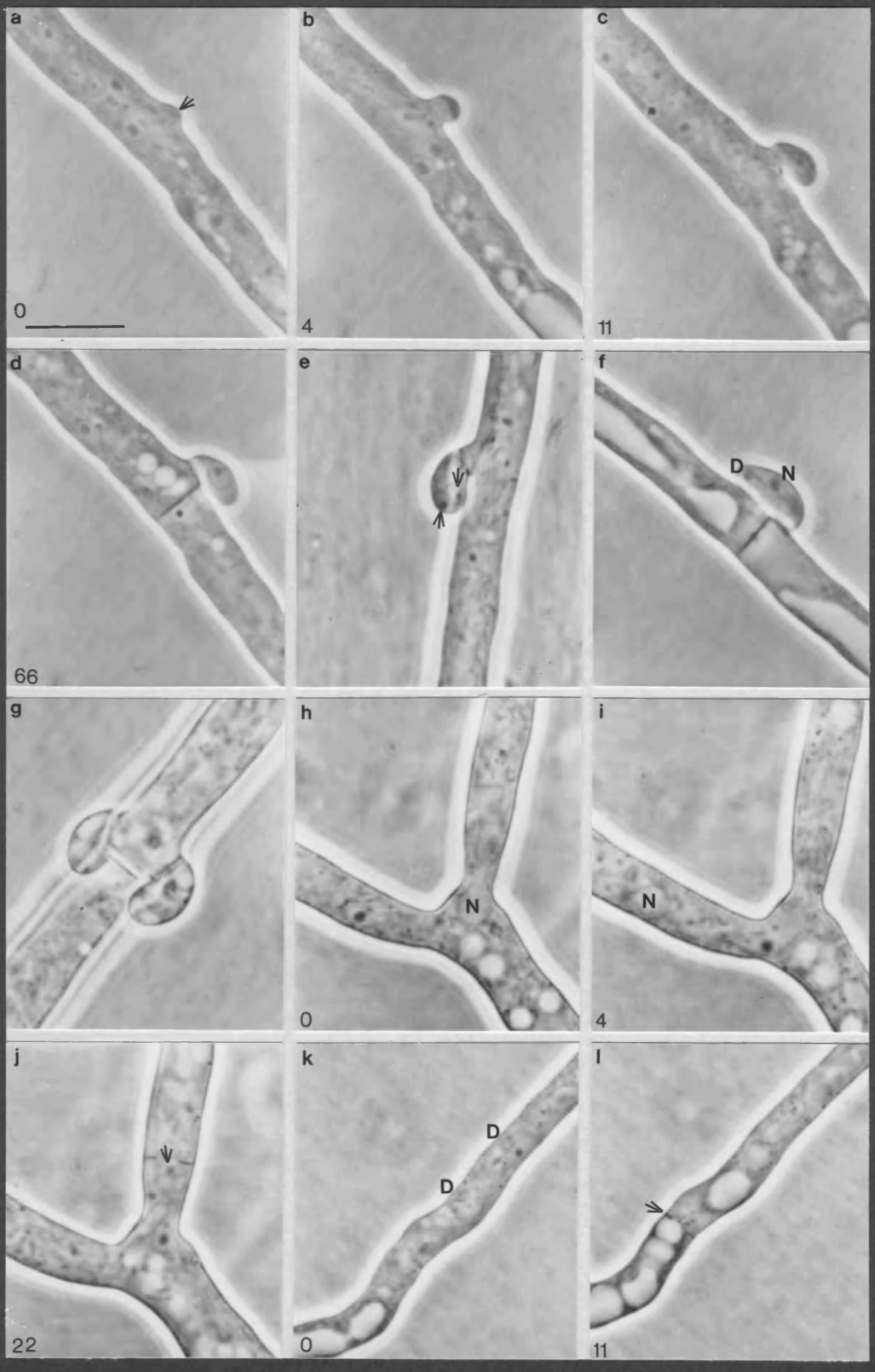
86

motion whilst fading from view. The whole nucleus then faded for up to 10 min, after which smaller globular nuclei, presumably division products, became visible. These exhibited jerky to and fro movements which gradually ceased as they enlarged and assumed the more irregular shape and slower movements of undivided nuclei. However, some division products with minute nucleoli failed to enlarge during the first hour after division, suggesting that they might have been defective.

About 15 min after the onset of division, a dolipore septum began to form across the fusion pore, closure being completed after about 10-15 min. However, this occasionally failed to occur and in relatively long H-bridge fusions consecutive septation could follow nuclear aggregation and division at both ends of the bridging hypha.

A similarly-timed sequence of nuclear aggregation and division, followed by septation, regularly occurred in extending heterokaryotic and homokaryotic apices (Fig. 8.5) and at sites where branching had occurred from intercalary compartments. In young secondary mycelia, concomitant formation of clamp connections regularly occurred on wider hyphae, but after approximately 24h in the microculture chamber, most hook cells failed to fuse with the subtending hypha. Hook cell initiation frequency later declined to zero, although division and septation proceeded as before. Hook cells of true and pseudoclamp connections were observed containing one of the following: a single post-division nucleus (Fig. 8.5d); a pair of post-division nuclei (Fig. 8.5e); a single undivided nucleus, or one of each (Fig. 8.5f).

Fig. 8.5. Nuclear division in P. velutina which does not follow hyphal fusion. (a-d) Development of hook cell during clamp connection formation in a heterokaryotic apical compartment. Hook initiation (arrowed) occurred adjacent to an aggregation of nuclei. Division of members of this aggregate was followed by migration of a daughter nucleus into the hook cell (c) followed by septation (d). (e) Hook cell of heterokaryon at onset of septation with a pair of daughter nuclei (arrowed). (f) Septate hook cell of a heterokaryon containing an undivided nucleus (N) and a daughter nucleus (D). Hook cell fusion failed to occur thus forming a pseudoclamp connection. (g) Paired pseudoclamp connection in which neither hook cell fused with the subtending homokaryotic hypha. (h-j) Nuclei (N) in early stages of division in a branched heterokaryotic hyphal apex. Septation (j) occurred at both sites commencing where the first nuclear division took place (arrowed). (k,l) Nuclear division products (D) in homokaryotic apex and septation (arrowed) at the division site. Bar markers represent 10μm. Time (min) is indicated in the lower left-hand corner of (a)-(d), (h)-(j) and (k),(l).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EVENTS AT AND AFTER NON-SELF FUSION

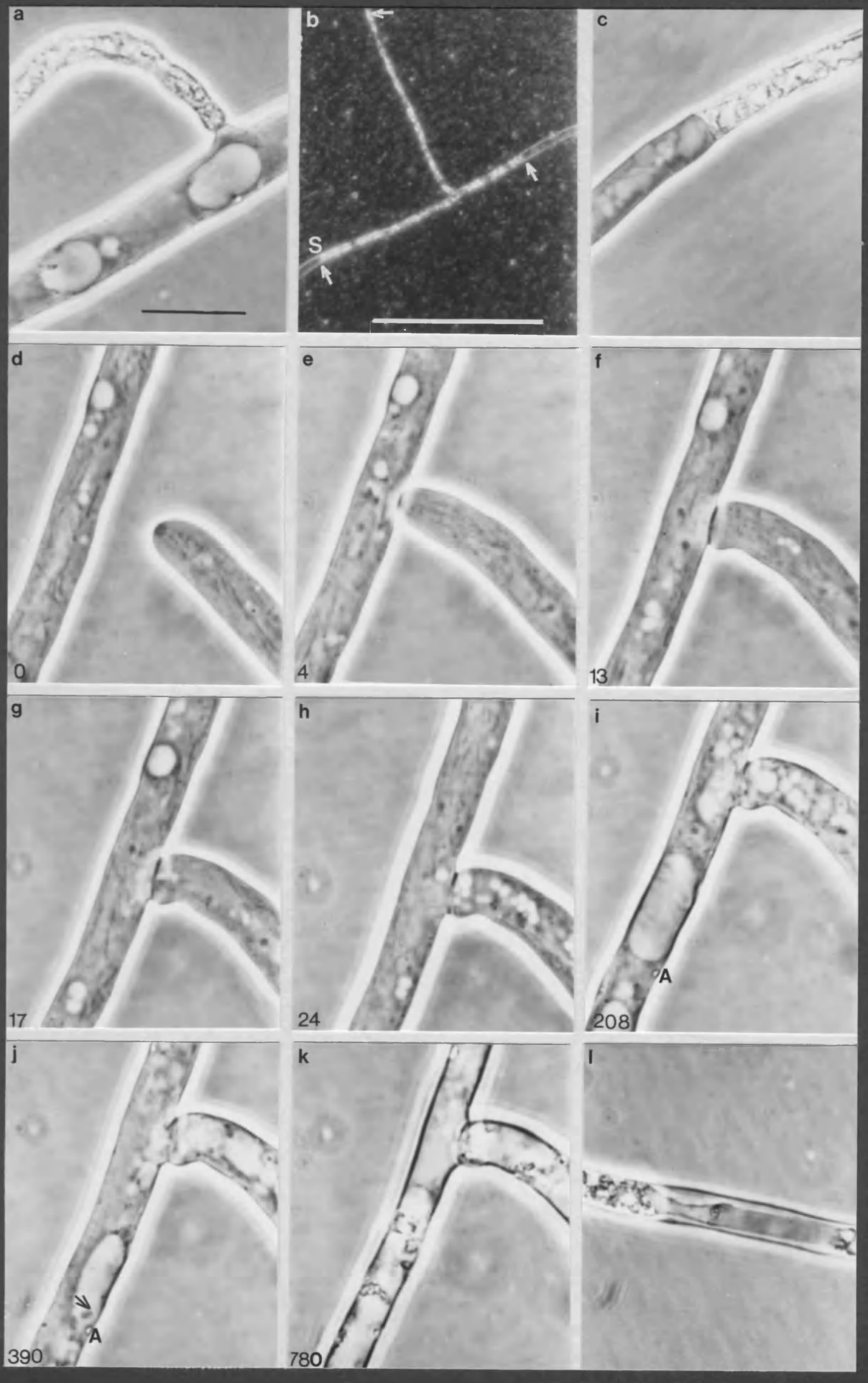
Fusions between hyphae belonging to different mating-type incompatible homokaryons exhibited a similar behavioural sequence to that in self fusions. However, those between different heterokaryons and between mating-type compatible homokaryons departed radically from this pattern.

Fusion between heterokaryons.

These were characterized by the rapid development, after incomplete opening of the fusion pore (see below), of a lytic reaction. Usually this reaction affected both participating compartments equally, sometimes spreading to two or three immediately neighbouring compartments, but rarely it was markedly unilateral (Fig 8.6). Unilateral reactions were characterized by very rapid ($\leq$1min) cytoplasmic coagulation in one of the participating compartments before the fusion pore was visible or when its presence could only be inferred by the movement of granules into a recipient hypha (Fig. 8.6a). Enlargement of the fusion pore usually occurred until it was clearly visible, but expansion to the extent seen in self fusions was never observed.

The fusion pores were traversed by mitochondria, cytoplasmic granules and vacuoles, but nuclei were not seen to migrate through them except, occasionally, as an apparent result of equilibration of cytoplasmic pressure. Within 5 min of opening of the fusion pore, nearby cytoplasmic activity in the recipient compartment, as manifested by the motion of organelles, temporarily subsided before initiation of a phase of progressive vacuolation (Fig. 8.6h-j). Dark globular structures containing oscillating darker fragments occurred transiently within the

Fig. 8.6. Non-self fusion sequence between P. velutina heterokaryons. (a) Unilateral rapid lysis occurring in fusing apex after homing and opening of a very small pore. (b) Bilateral lysis whose extent (arrowed) is restricted to one recipient and one fused apical compartment (dark ground optics). Septum (S) partitioning living and lysed recipient hyphal compartments is shown enlarged in (c). (d-k) Tip-to-side fusion showing progressive bilateral lysis and vacuolation (h-j) occurring after incomplete pore opening (g) resulting in virtually empty hyphal shells (k). This was accompanied by transient globular dark structures occurring within vacuoles (arrowed) and localized wall accretion (A). (l) Collapsed plasma membrane following unilateral rapid lysis in a fused apical compartment. Bar markers represent 10μm (a), (c-l) and 50μm (b) respectively. Time (min) is indicated in the lower left-hand corner of (d)-(k).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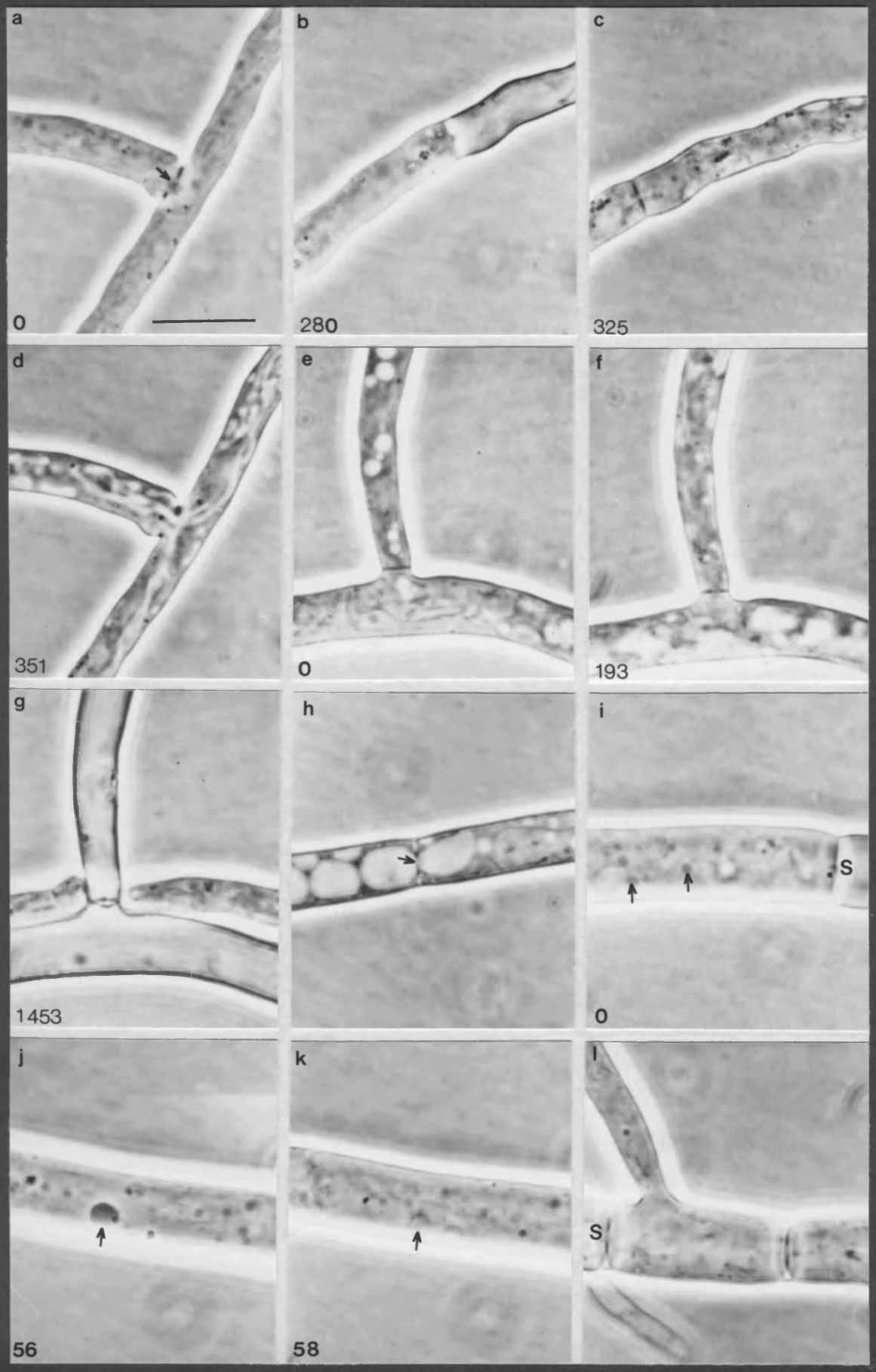
vacuoles (Fig. 8.6j), apparently originating by invagination of surrounding cytoplasm. As vacuolation proceeded, localized wall accretions (Fig. 8.6i,j) became visible throughout the lytic region, and were associated with a marked increase in refractility. After about 10h, the fusion compartments were reduced to virtually empty shells (Fig. 8.6k), sometimes with regions of collapsed plasma membrane (Fig. 8.6l), and were sometimes invaded by intra-hyphal hyphae.

Fusion between mating-type compatible homokaryons

The majority of these fusions resulted in either a rapid lytic reaction of the type observed between heterokaryons, or in delayed lysis. Rapid lysis predominated in early encounters between young mycelia, whereas delayed lysis was characteristic of later encounters. The behaviour pattern preceding delayed lysis paralleled that in self fusion and involved opening of the fusion pore, followed by almost synchronous nuclear division in the pore region and, in most cases, formation of a dolipore septum across the region. Vacuolation of the fusion compartments eventually followed, according to the pattern described previously, and was usually complete within 10h of septal closure.

In six cases the self fusion behaviour pattern was followed by rounds of septal erosion and nuclear migration, after which the fusion compartment and several neighbouring compartments could undergo vacuolation and lysis as described previously (Fig. 8.7a-g). Septal erosion began 2-3h after nuclear division in the pore region and with the pore septum (Fig. 8.7e,f) if present. After erosion, which always left a prominent septal rim (Fig. 8.7c,f-h), nuclear migration occurred,

Fig. 8.7. Events after mating-type compatible non-self fusion of homokaryons. (a) Donor nucleus (arrowed) passing through an incompletely opened fusion pore from an apical to an intercalary recipient compartment. After an increase in apparent turgor pressure within the recipient compartment, its rearward septum (b) was eroded (c). During the ensuing period of nuclear migration, elongated vacuoles entered the recipient compartment via the fusion pore (d). (e-g) Sequential occurrence of septation (e), septal erosion (f) and lysis (g) in a single fusion compartment. (h) Vacuole constricted during passage through an incompletely eroded septum (arrowed) in ex-homokaryon. (i) Aggregation of many migrant nuclei, e.g. visible nucleoli (arrowed), near an intact septum (S). (j) Member of the nuclear aggregate in an early stage of disintegration (arrowed) after nucleolar expansion. (k) 2 min later its contents have faded leaving a spherical membrane (arrowed). (l) Pair of eroded septa in a migration hypha. At the onset of nuclear migration, 21h previously, only septum (S) of the pair was present. Bar marker represents 10μm. Time (min) is indicated in the lower left-hand corner of (a)-(d), (e)-(g) and (i)-(k).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but its extent was mostly restricted to six to ten compartments by intact septa. Aggregation of many nuclei behind an intact septum was followed by their individual disintegration over a period ≥2h (Fig. 8.7i-k). Disintegration began with expansion of the nucleolus until the entire nucleus became spherical and dark (Fig. 8.7j). Approximately 2 min later, the contents faded completely, leaving behind a spherical membrane (Fig. 8.7k) which gradually disappeared. Nuclear migration had ceased, in all cases examined, within 24h of initial septal erosion. The hyphae in which migration had occurred contained sparsely distributed nuclei and both intact and eroded septa (Fig. 8.7l). Some of these septa had formed after the onset of migration.

DISCUSSION

Since hyphal extension preceding fusion was often at 90° to a lateral wall, Buller (1931) initially considered that it was as if a chemotropic stimulus was being produced from a site in the lateral wall. Such an attraction could culminate in tip-to-side fusion but Buller (1933) then re-interpreted all hyphal fusions as being of the tip-to-tip type, conceding that peg formation was sometimes barely visible. Using Burgeff's (1924; cited by Buller, 1933) terminology as originally applied to sexual reproduction in the Zygomycotina, it was suggested that the fusion sequence was initiated by action at a distance (telemorphosis) not exceeding 10-15μm (Burnett, 1976). This stimulated tip formation from lateral walls and was followed by a phase of zygotropism in which the apices grew towards each other before fusing.

Two theories were proposed to account for these observations. One postulated the production of a single diffusible chemical from a growing hyphal tip which initiated both the telemorphotic and zygotropic responses (Raper, 1952). The other suggested that fusion between tips was a result of overlap of haloes of low concentration of staling products (Park, 1961, 1963; Robinson & Park, 1965; Park & Robinson, 1966). The latter theory fails to account for initiation of hyphal tips in tip-to-side confrontations, and neither theory accounts for the mutual repulsion between main hyphal tips mentioned by Burnett (1976) and observed during the present study.

Buller's (1931) original interpretation that hyphal fusion was preceded by a chemotropic stimulus emanating from a potentially receptive site in a lateral wall was supported by the present and other recent studies. These studies, which involved hyphae interacting on cellophane membranes and at low nutrient levels, demonstrated that tip-to-side fusions do occur (Watkinson, 1978; Aylmore & Todd, 1984a; Todd & Aylmore, 1985).

In P. velutina, the distance over which redirected apical extension occurred indicated a more far-reaching stimulus than had been previously implied. However, such long range curvatures were only rarely observed in semi-solid culture media suggesting that relatively high concentrations of a putative chemical signal were accumulating around hyphae constrained in a monolayer between a glass coverslip and a cellophane membrane.

Site-directed hyphal curvature is also implicated in homing responses of hyphal tips to arthroconidia and basidiospores which have been observed in several Basidiomycotina (Bistis, 1970; Kemp, 1970, 1977, 1980b; Fries, 1981, 1983). In

the case of basidiospores, this is preceded by induction of a spherical germ vesicle by the nearby presence of hyphae of the same or a closely related species. These hyphae then home towards and attach to the germ vesicle (Fries, 1981, 1983).

The mechanism underlying curvatures remains obscure however. The behaviour of the Spitzenkörper might suggest some role in detection of a stimulus from a receptive site, but since displacement of this body is a general feature before hyphal curvature (Girbardt, 1955, 1957; Grove, 1978), cause and effect are difficult to resolve. It may be significant that those fungi which possess a Spitzenkörper (Ascomycotina and Basidiomycotina) are just those in which hyphal fusion occurs most readily.

Nuclear behaviour and septation during self fusion exhibited markedly different patterns from those reported previously for C. versicolor and S. commune (Aylmore & Todd, 1984a; Todd & Aylmore, 1985). Thus the nuclear replacement reaction was not observed, and, except in clamp connections, septation was always directly across the fusion pore, which coincided with the site where numerous nuclei divided. In C. versicolor and S. commune, nuclear division and septation also spatially coincided with each other, but not with the fusion pore. Nevertheless, the capacity to undergo the same process of nuclear degeneration which precedes replacement in C. versicolor and S. commune was exhibited, after mating-type compatible fusions, by nuclei whose migration was blocked by an intact septum. These differences in behaviour are probably associated with less strict control of nuclear numbers (up to 80 have been observed) in the holocoenocytic compartments of P. velutina, compared with that in monokaryons and dikaryons. This

probability is enhanced by recent observations with another basidiomycete, Chondrostereum purpureum, in which typical nuclear replacement reactions have been observed, together with regularly binucleate compartments in the heterokaryon (A.M. Ainsworth, unpublished).

The other major departure from the behaviour of C. versicolor and S. commune was in the rapidity of onset of the lytic reaction. In these latter species, although the macroscopic effects of hyphal rejection are easily observed in plate culture, fusions between dikaryons grown under the same conditions as described herein, all resulted in the same behavioural sequence as that seen in self fusions (Aylmore & Todd, 1984a; Todd & Aylmore, 1985). This implies that the rejection responses are slow to develop and/or precluded by the nuclear replacement reaction.

The occurrence of rapid or delayed lytic interactions between mating-type compatible, but not between incompatible, homokaryons correlated with behaviour during interactions in plate culture on 2% MA (Chapter 5). These responses were also similar to the "lethal" cytoplasmic reactions which have been reported to follow homing to arthroconidia and basidiospores in Coprinus and Leccinum respectively (Kemp, 1977; Fries, 1981, 1983). However, in this case the reactions follow fusion with spores of closely related species, but not with the same species. Since at least the spores would be homokaryotic, it is likely that lethal reactions following conspecific non-self fusions would either be delayed, or overridden by mating-type compatibility.

A further parallel, particularly evident in the unilateral rapid cytoplasmic coagulation reaction shown in Fig. 8.6a, is with hyphal interference, whereby hyphal contact, but not fusion, between different species results in lysis of one or both participant compartments. In Ascobolus crenulatus (Ascomycotina) contacted by Coprinus heptemerus, increased refractility and vacuolation occurs (Ikediugwu & Webster, 1970; Ikediugwu, 1976) which seems to be fundamentally similar to that in non-self interactions of P. velutina (see Aylmore & Todd, 1986b).

APPENDIX i COMPOSITION OF MEDIA

2% MA - 2% w/v malt extract agar

Spray malt extract (Munton & Fison)	$20gl^{-1}$
Agar ("Labm" No. 2)	$20gl^{-1}$
Distilled water to give final volume of	1 litre

The medium was autoclaved at 121°C for 15 minutes and dispensed at *ca.* 15ml or *ca.* 50ml per 9cm or 14cm diameter non-vented disposable plastic Petri dish respectively.

2% MAN - 2% MA with the addition of $0.1gl^{-1}$ Novobiocin (Sigma) before autoclaving to suppress bacterial contamination.

2% MANB - 2% MAN with the addition of $2mgl^{-1}$ Benomyl (Benlate, Du Pont) before autoclaving to suppress fungal contaminants, e.g. *Trichoderma*.

APPENDIX ii NAMES AND AUTHORITIES OF FUNGAL SPECIES MENTIONED IN THE TEXT

ZYGOMYCOTINA

Rhizopus stolonifer (Ehrenberg ex Fries) Lind

ASCOMYCOTINA

Ascobolus crenulatus Karsten

Chromocrea spinulosa (Fuckel) Petch

Cochliobolus heterostrophus (Drechsler) Drechsler

Emericella nidulans (Eidam) Vuillemin

Eurotium amstelodami Mangin

Neurospora crassa Shear & Dodge

Neurospora tetrasperma Shear & Dodge

Ophiostoma ulmi (Buisman) Nannfeld

Podospora anserina (Rabenhorst) Niessl

Pyricularia oryzae Cavara

Saccharomyces cerevisiae Hansen

Sclerotinia trifoliorum Eriksson

BASIDIOMYCOTINA

Agaricus bitorquis (Quélet) Saccardo

Agaricus macrosporus (Möeller & Schaeffer) Pilát

Agrocybe cylindracea (de Candolle ex Fries) Maire

Armillaria mellea (Vahl ex Fries) Kummer

Athelia rolfsii (Curzi) Tu & Kimbrough

Bjerkandera adusta (Fries) Karsten

Chondrostereum purpureum (Persoon ex Fries) Pouzar

Clitocybe truncicola Peck

Coniophora arida (Fries) Karsten

Coniophora puteana (Schumacher) Karsten

Coprinus bisporus J. Lange

Coprinus cinereus (Schaeffer ex Fries) S. F. Gray

Coprinus disseminatus (Persoon ex Fries) S. F. Gray

Coprinus heptemerus M. Lange & A. H. Smith

Coprinus macrorhizus f. microsporus Hongo

Coprinus patouillardii Quélet

Coprinus radiatus (Bolton) Fries

Coprinus sterquilinus Fries

Coriolus versicolor (Fries) Quélet

Coriolus zonatus (Nees) Quélet

Cyathus stercoreus (Schweinitz) de Toni

Fomitopsis cajanderi (Karsten) Kotlaba & Pouzar

Heterobasidion annosum (Fries) Brefeld

Hirneola auricula-judae (St. Amans) Berkeley

Hirschioporus abietinus (Dickson ex Fries) Donk

Hymenochaete tabacina (Sowerby) Léveillé

Hyphoderma mutatum (Peck) Donk

Lenzites betulina (Linnemann ex Fries) Fries

Mycena galopus (Persoon ex Fries) Kummer

Peniophora reidii Boidin & Lanquetin

Phaeolus schweinitzii (Fries) Patouillard

Phanerochaete laevis (Fries) Eriksson & Ryvarden

Phanerochaete velutina (Fries) Karsten

Phellinus weirii (Murrill) Gilbert

Phlebia radiata (Fries)

Phlebia rufa (Fries) M. P. Christiansen

Piptoporus betulinus (Fries) Karsten

Pleurotus ostreatus (Jacquin ex Fries) Kummer

Polyporus brumalis Fries

Polyporus ciliatus Fries

Polyporus palustris Berkeley & Curtis

Psathyrella candolleana (Fries) Maire

Psathyrella coprobia (J. Lange) A. H. Smith

Pseudotrametes gibbosa (Persoon) Bondartzev & Singer

Psilocybe coprophila (Bulliard ex Fries) Quélet

Schizophyllum commune Fries

Sistotrema brinkmannii (Bresadola) Eriksson

Stereum complicatum Fries = S. rameale (Schweinitz) Burt

Stereum gausapatum (Fries) Fries

Stereum hirsutum (Willdenow ex Fries) S. F. Gray

Stereum insignitum Quélet

Stereum ochraceo-flavum (Schweinitz) Ellis = S. rameale (Persoon) Fries

Stereum rugosum (Persoon ex Fries) Fries

Stereum sanguinolentum von Albertini & Schweinitz

Stereum subtomentosum Pouzar

Stereum sulphuratum Berkeley & Ravenel

Thanatephorus cucumeris (Frank) Donk

Typhula idahoensis Remsberg

Typhula ishikariensis Imai

Typhula micans (Fries) Berthier

Typhula trifolii Rostrup

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